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Twenty-one years of the double helix

WE celebrate in this issue the twenty-first anniversary of the appearance of J. D. Watson and F. H. C. Crick's short paper *A Structure for Deoxyribose Nucleic Acid* in *Nature*. A few will be offended by the equating of the twenty-first birthday with the process of coming of age when in the past few years the age of maturity has dropped to eighteen in many countries. We can do little for them. Rather more, with good cause, will say that there were earlier milestones than 1953 which marked the turning point in the fortunes of molecular biology. 1943 was a key year, with Avery's work on pneumococcus DNA at the Rockefeller Institute and Beadle and Tatum's on *Neurospora* at Stanford. As Macfarlane Burnet put it in *Genes, Dreams and Realities*, genetics was by these experiments directly implicated in biochemistry through micro-organisms. Others will also have their favourite years during which a quickening occurred, but few would carp at the Watson-Crick announcement being given pride of place as a starting point of something absolutely new and some (though perhaps not many physical scientists—either through ignorance or conviction) might go along with Medawar in calling it "the greatest achievement of science in the twentieth century".

We have tried to give a varied view of the history, present scene and prospects in molecular biology, from Chargaff's iconoclastic essay to Gurdon's report on the exciting developments in introducing macromolecules into living cells. One thing has become clear. A central contribution of the magnitude of Watson and Crick's stirs up the culture of science to a quite remarkable degree, and it is a change in the cultural climate, perhaps more even than the actual discovery itself, which breathes new life into science. Suddenly there are all sorts of new questions of history, philosophy, method and attitude to occupy the minds of the best scientists, along with the starker scientific questions and the less edifying ones of funding, organisation and priorities. Cultural revolutions in science are rare and immensely stimulating to those touched by them.

From the historical point of view an interesting aspect is the way in which the structure came to be accepted. As Brenner puts it, there was initially only a small band of believers and a positive effort had to be made to convince the biological community both of its correctness and profound relevance. This conforms to a classical view of a seminal idea's acceptance—first accepted by the few, later sweeping all before it. Contrast it with a more recent revolution in the earth sciences. There a whole army was waiting to pick up the central dogma of plate tectonics and make it their own. Was Watson-Crick the last great scientific idea that needed missionaries? It

seems unlikely. Although the number of scientists waiting for things to drop into their lap is greater than ever before, there is no sign that the community is any more sensitive to revolutionary ideas, however plainly presented.

The philosophical debates stirred up by molecular biology almost seem like a re-run of some of the issues generated by the explosive advances in physics early in this century. In the 1920s Eddington was talking in *The Nature of the Physical World* of his two tables; one a commonplace, substantial table—a "thing"; the other a scientific table, pervaded by fields of force and electric particles—an "influence". He clearly distinguishes between a "familiar world" and a "scientific world revealed by physics". The present debate in biology between reductionists and holists seems to resurrect all these old distinctions. Is a biological system more than the sum of the properties of its parts? For the philosopher, molecular biology has posed some fine questions.

Yet however stimulating molecular biology has been to historians and philosophers, the most fascinating debate has been among scientists themselves. Has the influence of molecular biology been good on biology as a whole? You will find both positive and negative answers in the articles that follow. Has molecular biology really done nothing for medical science as Burnet and many others would claim? Has it so distorted the funding of medicine that competent medical science is at risk, and has it practically destroyed certain fields of research, such as human nutrition, to which it is very difficult to attract first-rate talent? Is the pursuit of a Nobel prize through molecular biology the ultimate aim of every budding biologist? So the muttering goes, both in public and in private. These are not questions for quick answers; they are ones, however, that those outside biology should be aware of, for they represent the very central issues of science and science policy and, who knows, may crop up anywhere else at very short notice. It only needs the right sort of paper in *Nature* to start it all off.

100 years ago



DR. LYON PLAYFAIR, C.B., has given notice that, on the House of Commons going into committee on the Education Estimates, he will call attention to the deficient ministerial responsibility under which the Votes for Education, Science, and Art are administered, and will move for a Select Committee to consider how such ministerial responsibility may be better secured. We believe that Dr. Lyon Playfair's views are strictly in accordance with those of the best scientific men of the country, namely, that the only satisfactory way of dealing with the subject will be by the appointment of a Minister for Education, Science, and Art.

From *Nature*, 9, 511, April 30, 1874.



Should scientists be seen and not heard?

In recent years there has been a marked change in the way the British government is advised about scientific affairs. John Gribbin discusses this change, and the position of science in industrialised society today, in the light of the recent appointment of Dr Robert Press to coordinate the government's scientific advice.

WITH the departure of Sir Alan Cottrell to become Master of Jesus College, Cambridge, the post of Chief Scientific Adviser to the government is not to be filled from the outside world of science. Sir Alan had held his post with a grade of second secretary in the Cabinet Office, and as such ranked above almost all the scientific advisers in the various departments and ministries (the exception being Sir Hermann Bondi at the Ministry of Defence). Responsibility for coordinating scientific advice within the Cabinet Office now rests with Dr Robert Press, who as Deputy Secretary (Science and Technology) holds the same rank as most of his colleagues who are the advisers to the individual departments.

Dr Press will no doubt carry out his new role as well as anyone but the change is bound to be seen by many as a decline in the status of the post; Dr Press has also commented that he will be continuing with his previous work—chiefly concerned with nuclear affairs—so it seems that the post is not regarded as a full time occupation. And the decline of the post of Chief Scientific Adviser to the government cannot even be said to be sudden. Sir Alan himself was in many ways in a less powerful position than his predecessor, Sir Solly (now Lord) Zuckerman, and was never, for example, the Chief of the Scientific Civil Service. The latest move could well be seen as a sign that the previous situation, under Zuckerman, has now been reversed.

This is a new situation which deserves particularly close attention since so few members of the government—or indeed of either House—have any training in scientific or engineering matters. In the past, it has certainly proved possible for an all-powerful Civil Service to advise and persuade governments without taking into account the best scientific considerations; this may not happen now, but it would be much less likely to happen if there was, once again, a man in the centre with the experience and personality to take a firm grip.

No doubt the argument will be put forward from some quarters that there is no suitable man to hold such a position, and that therefore the government has been forced into the present situation. That argument carries some weight; there are always people to be found with forceful personalities and a desire to tell the government what to do about science but alas that is not sufficient qualification. What is needed is someone from the scientific community who is both well informed and respected by that community, but who has the strength of character to deal firmly with the community when necessary.

Another argument which was put forward after the announcement of Dr Press's appointment was that it is in line with the Rothschild proposals for the organisation of

civil science in Britain. Now that there are Chief Scientists in all the essential departments, so the argument runs, there is no need for anything more than a coordinator in the Cabinet Office. That argument, however, seems to rest on a singular interpretation of the Rothschild report.

But outside the immediate issue, the question of the place of science in society is even more disturbing. At present there is an urgent and growing need for science to be used constructively by governments; there is a strong likelihood that the present situation will be no different in practice from the situation with Sir Alan Cottrell as Chief Scientific Adviser, but there is very little chance of changes being made in the required direction. In part, to be sure, the need is for a cleaning up of the mess made by the misuse of science in the past. That, however, makes the present need for correct use of science no less urgent. Yet there seems to be a great public apathy about science, at least in the industrialised nations of the world.

There is a need to provide scientific education in the broadest sense, to get the scientific message across to the general public and to the rank and file Members of Parliament, if not to the government itself. There is at present a bizarre situation in which there is concern at all those levels that the few millions of pounds worth of aid which Britain is providing for drought-stricken regions of Africa may be inadequate, and yet there is no move to provide a few tens of thousands of pounds for research into the causes of such droughts (see *Nature*, 248, 466; 1974). Seldom can there have been a more straightforward example of prevention (if possible at all) being better than cure; there must be many other cases where foresight and the correct application of current scientific knowledge would produce enormous benefits.

Part of the blame for the confusion and apathy in the public mind must be laid at the door of the Press. There were only a few brief paragraphs in the British national papers commenting on the new situation in the Cabinet Office, and at least one responsible newspaper carried an incorrect and misleading account which largely obscured the real significance of the situation. Members of both the Royal Society and the Institution of Mechanical Engineers have recently expressed concern about the failure of scientists to get their message across. If this concern is to be turned into something more constructive, those august bodies might well be advised to investigate ways of encouraging the spread of the scientific message through the journalistic medium.

It is, however, less easy to see an obvious immediate solution to the problem of providing the right kind of scientific advice to the government. If there are serious objections to placing too much scientific authority in the hands of one man, there is one obvious alternative which has been touted, and that is to expand the role and responsibilities of the Advisory Board for the Research Councils (ABRC). This has superficial appeal. Sir Alan was, of course, represented on the ABRC and Dr Press will be too. So the channel of communication is there and might be strengthened into something more without great difficulty.

But government by committee is always undesirable and advice by committee hardly less so. Since the role of Chief Scientific Adviser, which has now disappeared, was largely one of assessing priorities, it is difficult to see how the work could be delegated and shared out. Someone must see everything in order to assess the priorities, and in that case the committee is redundant. There seems no easy solution; but the present situation is far from satisfactory. At a time when the government is faced with questions of greater scientific complexity and urgency than ever before in peacetime it is disturbing to find the scientific community asking: Where is the voice of science and engineering in government?; it is perhaps even more disturbing to guess the likely public response to such a question: We don't know and we don't care.

international news

EACH year in the United States between 100 and 1,000 people undergo a highly controversial surgical operation which consists of the destruction of tiny portions of their brain. Popularly known as psychosurgery and designed to alter behaviour, the operation has become the centre of a bitter public debate over its legal and ethical implications, as a result of which the federal government is cautiously moving toward adopting a set of recommendations and regulations for controlling the technique.

The latest move is that staff members of the National Institute of Mental Health (NIMH), the agency which funds the bulk of government-supported psychiatric research, have sent a report to the Assistant Secretary for Health listing a set of recommendations for consideration as official government policy. As set out in a memorandum signed by the Director of the NIMH, Bertram S. Brown, and prepared with the help of a panel of distinguished outside experts, the suggested regulations would outlaw some of the more controversial applications of psychosurgery—its use on children, prisoners and incarcerated mental patients—but they stop well short of calling for a complete ban on the technique.

The controversy generated by psychosurgery has stemmed largely from charges that the technique is nothing more than a dressed-up version of lobotomy, the surgical operation popularised in the 1950s which has since been discredited, leaving thousands of people with impaired mental function. Supporters of the technique argue, however, that it is an acceptable form of therapy for severe behaviour disorders which have not responded to more conventional psychiatric treatment.

Unfortunately, the debate has not been helped by the fact that many of the psychosurgery operations carried out in the United States have been performed with hopelessly inadequate follow-up procedures, so that it is difficult to form any conclusions about the efficacy of the technique. As the NIMH memorandum puts it, "inadequacy of pre- and post-operative behavioral and psychological testing, lack of long term follow-up of patients, and general inadequacies of clinical and behavioral reporting characterise much of the published literature."

On the other hand, critics of psychosurgery have managed to cite examples in which psychosurgery operations are

Rules for psychosurgery

Colin Norman, Washington

Brown: signed memo



alleged to have resulted in severe damage to the intellect of patients and they have brought up passionate arguments to suggest that the operation has been used to alter the behaviour of emotionally disturbed children for the convenience of their parents.

One incontrovertible aspect of the operation is that its effects are irreversible because it involves the destruction of brain tissue which is not regenerated. In that case, why did the NIMH not recommend at least a moratorium on the technique until some of the more serious charges that have been levelled against the operation are cleared up?

The memorandum gives three reasons. First, such a recommendation "would constitute an unprecedented federal prescription of the parameters of permissible and impermissible surgery for the medical profession". (The NIMH staff did not feel themselves to be under such a constraint in recommending that the technique should not be used on children, prisoners and involuntarily detained mental patients, however.) Second, since there is no precise definition of psychosurgery, the memorandum suggests that a moratorium would be rendered ineffective because the operation could be performed under the guise of treatment for epilepsy and other neurological disorders. And third, the proposed regulations would at least amount to a partial moratorium on the most controversial forms of psychosurgery.

In short, the memorandum suggests regulations "with the intent of providing the maximum possible protection for potential psychosurgery candidates without unduly inhibiting practice for those cases which, judged by our present standards and knowledge, appear to require psychosurgery for relief of extreme mental illness or behaviour disorders". So the NIMH at least accepts some of the arguments for psychosurgery.

Perhaps the most important recommendation is that psychosurgery should be regarded as strictly experimental and not a form of therapy. Such a designation would slap a number of controls on use of the technique, such as the development of comprehensive research protocols to ensure that maximum scientific value is gained from each operation. It would also mean that psychosurgery should only be carried out in hospitals attached to universities and "every effort must be made to ensure that all reasonable alternative therapies . . . are attempted to an adequate extent before resorting to psychosurgery".

The NIMH is also recommending that a widespread effort be initiated by the federal government to obtain more precise information about the results of psychosurgery operations performed in the past, the idea being that such information can later be used as a basis for more permanent guidelines governing the future use of the technique.

The memorandum is now being reviewed by Charles C. Edwards, Assistant Secretary for Health, but whatever finally becomes of it, the federal government can only directly control those psychosurgery operations performed with government money. The control of such operations carried out in hospitals and universities independently of federal funds lies outside the direct jurisdiction of the Department of Health, Education and Welfare.

But at least the widespread public controversy which has been generated in the past couple of years, chiefly through a court case which halted the use of psychosurgery on an involuntarily detained mental patient last year and a bruising public inquiry conducted by Senator Edward M. Kennedy's health subcommittee, should make it more difficult for the operation to be carried out with inadequate controls.

Pure science and the energy problem

Miranda Robertson, Chicago

MUCH the largest contribution of the Argonne National Laboratory to the problem of developing a new energy technology is its experimental liquid-metal fast-breeder reactor, and nuclear energy will continue to account for nearly half the total research expenditure of the laboratory. But recent shifts in the emphasis of federal funding are reflected in the expansion of research on a wide range of alternative and accessory approaches to the problem. At the same time, the laboratory (which is controlled by the United States Atomic Energy Commission) is beginning to feel the effects of a general movement to involve basic scientists in applied research, as interpreted by its new director, Dr Robert G. Sachs, now at the end of his first year at Argonne. A vigorous proponent of the view that fundamental scientists represent a national resource to be supported for reasons of enlightened self-interest, he believes that the time has now come to draw on that resource for the answers to a national problem. He also believes that a degree of mission-orientation need detract from nobody's fundamental research pursuits. The effects of both federal and local policy are nowhere better illustrated than in the birth of a new research project on solar energy in which Argonne personnel are collaborating with Dr Roland Winston from the Enrico Fermi Institute at the University of Chicago, where Dr Sachs holds a chair.

The outcome of this joint enterprise could be the development of a much more efficient solar energy collector than could be envisaged with known techniques; but it began as pure research on high energy physics. The problem to which the Winston advice was originally addressed was that of collecting Cerenkov radiation for the detection of rare electrons resulting from the β decay of a λ particle. The essential feature of the device was that it should not miss any radiation from the reaction.

Eventually, by departing from the principles of imaging optics, which limit the possible degree of concentration of the intensity of solar radiation to a factor of three, he arrived at an ideal light collector in the form of a cylindrical paraboloid with a theoretical maximum degree of concentration of ten times. The device represents a new principle as far as modern physics is concerned, although Professor Ricardo Levi Setti, a colleague of Winston's in the physics department, has discovered identical

shapes in drawings of Descartes and in the individual eye-cups of the horse-shoe crab. What Levi Setti also recognised, however, was that as well as having an interesting past the device has an important potential future as a concentrator of solar energy.

The problem of concentrating sunlight is only one of the obstacles to the utilisation of solar power to generate electricity, but it is one of the principal stumbling blocks in using it to heat and cool individual homes. The exploitation of solar energy for this purpose is already feasible and as a result of vigorous advocacy in the past year there has been an increase in the allocation of research funds as well as the institution of tax incentives for the conversion of buildings from conventional heating and cooling systems. But although solar heating is comfortably within the capacity of existing types of collector, cooling, which is much less efficient and in some areas in the summer stretches electricity generation to the point of overload, is more marginal. The increased concentration factor of the Winston light collector may overcome the difficulty. Its other

important advantage is that it can be designed so as to obviate the need for tracking the movement of the Sun across the sky, thus greatly reducing the complexity and the energy cost of the system. Winston's first model for a roof unit consists of a series of collectors shaped as paraboloid troughs which can be aligned with the Sun's trajectory so as to collect light for an average of eight hours a day.

The promise of the device has been endorsed by the allocation, in mid-fiscal year, of some \$200,000 from the United States National Science Foundation and the Atomic Energy Commission for feasibility studies. Winston and his collaborators at the Argonne are now working on such questions as how much light is actually collected by an ideal light collector made from nonideal materials and functioning under nonideal conditions. One of the problems being tackled is the development of what amounts to a new meteorological dictionary: for example, 'overcast' might mean something very different in terms of collectable solar radiation than it means in the week-end weather report.

Solar energy in suspense *Colin Norman, Washington*

Although almost everybody agrees that solar energy is a splendid idea whose time has come, a bill designed to hasten commercial application of solar heating and cooling technology has fallen foul of a jurisdictional squabble in the United States Senate and its passage has been delayed for several months while no less than five different committees have staked their claims on the legislation.

Passed by the House of Representatives in mid-February, the bill is designed to provide federal support for the development and testing of solar heating and cooling devices in private homes, office buildings, factories, schools and various other buildings. The idea is that the National Aeronautics and Space Administration (NASA) would be given some money to let contracts with industry for development and mass production of solar heating and cooling units, while the Department of Housing and Urban Development (HUD) would be responsible for carrying out prototype demonstrations to see how well the units perform in everyday use.

After being approved by the House—by a margin of 248 votes to two—the bill was referred to the Senate Committee on Aeronautical and Space Sciences which made a few amendments and passed it on March 13. In the meantime, how-

ever, four other senate committees—Commerce, Labour, Interior and Banking, and Housing and Urban Affairs—all argued that the bill should come under their purview, so it was then referred to each of them separately.

The irony is that once the various committees have finished guarding their legislative jurisdictions, the bill which finally emerges will probably be similar to the version which was passed by the House, except for one important detail. The House bill would have provided \$50 million for the programme over the next five years, but the Senate Space Committee has suggested that NASA and HUD should each get \$5 million next year to carry out their parts of the enterprise and funding for future years should be left open for now.

As for the administration's attitude to the bill, Dr H. Guyford Stever, Director of the National Science Foundation and Science Adviser to the White House, has argued that although the demonstration scheme is a good idea, it is a little premature. The Administration has already proposed that \$50 million should be spent next year on solar energy research, and Stever reckons that it would be wise to wait until some of this effort bears fruit before rushing into a federally sponsored application programme.

The Strangelove scenario

Colin Norman, Washington

BY THE year 2000 there could be enough plutonium and enriched uranium flowing through the nuclear power industry in the United States to manufacture about 250,000 crude atomic bombs. The sheer magnitude alone is sufficient to conjure up Dr Strangelove scenarios of nuclear theft. But is it really feasible for a few terrorists to make off with several kilograms of the stuff, fashion it into a weapon and hold a city to ransom—or even to create a nuclear holocaust in the Middle East?

According to a book-length report prepared for the Ford Foundation's Energy Policy Project, the short answer is yes, unless the Atomic Energy Commission (AEC) improves its control over weapons-grade nuclear material and unless officials in the energy industry, many of whom are "only vaguely aware of the problem", are made to take effective (and costly) steps to safeguard the potential nuclear weapons that they handle every day. (*Nuclear theft: Risks and Safeguards*. Mason Willrich and Theodore B. Taylor. Ballinger Publishing Company, Cambridge, Massachusetts, 1974).

The study's conclusions are likely to provide some powerful ammunition to nuclear critics in the United States who have recently seized on the issue of weapons' proliferation as yet another club with which to beat the AEC and the nuclear industry, particularly with respect to plans to build a string of plutonium-producing fast-breeder reactors around the country. But they are unlikely to shake the Administration's unswerving commitment to nuclear power, and spokesmen for the AEC have already rebutted the report's chief conclusion by arguing that existing safeguards are sufficiently stringent to ensure adequate protection of public safety and national security.

Be that as it may, the study provides strong evidence to suggest that a crude atomic bomb could be manufactured in a clandestine laboratory by a few people with a relatively modest amount of nuclear know-how, about 10 kg of plutonium oxide, a substantial amount of chemical high explosive and "materials and equipment that could be purchased at a hardware store and from commercial suppliers of scientific equipment for student laboratories". A bomb made of highly enriched uranium (containing more than 90% uranium-234) or uranium-233 would be assembled a little differently from a plutonium bomb, but it could also be made relatively easily with a supply of

15 kg or more of fissionable material.

Although that may sound like fantasy to some weapons designers, the authors of the study point out that few experts have asked themselves the question: "What is the easiest way I can think of to make a fission bomb, given enough fission-explosive material to assemble more than one normal density critical mass?" And they cite an article in the *Encyclopedia Americana*, written by Dr John S. Foster, former Director of Defense Research and Engineering and an expert on nuclear weapons' technology, who states that "the only difficult part of making a fission bomb of some sort is the preparation of a supply of fissionable material of adequate purity: the design of the bomb itself is relatively easy".

Moreover, the two authors of the report have some impressive credentials in the nuclear weapons field to back up their analyses. One of them, Mason Willrich, now Professor of Law at the University of Virginia, was General Counsel of the United States Arms Control and Disarmament Agency, and the other, Theodore B. Taylor, worked on nuclear weapons design at the Los Alamos Scientific Laboratory and was Deputy Director of the Pentagon's Defense Atomic Support Agency. Taylor emerged a few years ago as the chief critic of the AEC's nuclear safeguards and has become a rather prominent thorn in the agency's flesh.

It is not difficult to predict the consequences if a crude nuclear weapon does end up in the hands of a terrorist organisation. The authors of the study point out, for example, that even a "small" nuclear device exploded underneath a large city skyscraper might kill as many as 50,000 people: a comparable explosion in the centre of a football stadium could lethally irradiate about 100,000. As for the injection of nuclear weapons into the Middle East, the political fallout would be devastating.

So, assuming the validity of the theory that a crude atomic weapon could be manufactured relatively easily, the crucial consideration is how easily can fissionable material be stolen from the nuclear power industry? Willrich and Taylor argue that although there is little risk at present that a terrorist could make off with a few critical masses of explosive material, the burgeoning growth of nuclear energy and the changing characteristics of power plants will greatly increase the chance of illicit diversion of the stuff. They suggest that unless the AEC adopts an improved safeguards system now, it may soon be too late.

The most vulnerable part of the fuel cycle to terrorist activities is the transportation of weapons-grade materials

between facilities, according to the study, and the greatest chances for diversion will come when large quantities of plutonium (in particular) are moved around the country. At present, the movement of plutonium is not substantial, but in the next few years it is likely to grow significantly.

Nuclear energy is now produced almost entirely by light water reactors which are fuelled with uranium, only slightly enriched with the uranium-235 isotope. The fuel itself is thus of no use as weapons material, but the reactors do produce plutonium, which is separated out from the spent fuel rods and stored. According to Willrich and Taylor, this plutonium is now adequately protected against theft, but in the late 1970s it will be recycled for use as fuel in light water reactors, which means that it will be transported from the storage facilities to the power plants. The inherent risk of theft will then be "considerably greater".

In the 1980s and 1990s, when the liquid-metal fast-breeder reactor (LMFBR) is likely to be introduced commercially, the availability of weapons-grade nuclear material will again increase dramatically. The LMFBR produces large quantities of plutonium, which will be extracted from the spent fuel rods and recycled for use as reactor fuel. Since the AEC has forecast that by the year 2000 breeder reactors could be generating up to 750,000 MW of electricity, Willrich and Taylor calculate that if all goes according to plan, about 3,000 truckloads of plutonium oxide would be shipped to LMFBR and light water reactor fuel fabrication plants every year.

And these figures are given added significance if the possibility that a small amount, insufficient to make a bomb but enough to make a radiological weapon, is taken into account. Since plutonium is highly toxic, and easily dispersed in the form of tiny airborne particles, it would be possible to pose a severe risk to a large population with only about 100 grams.

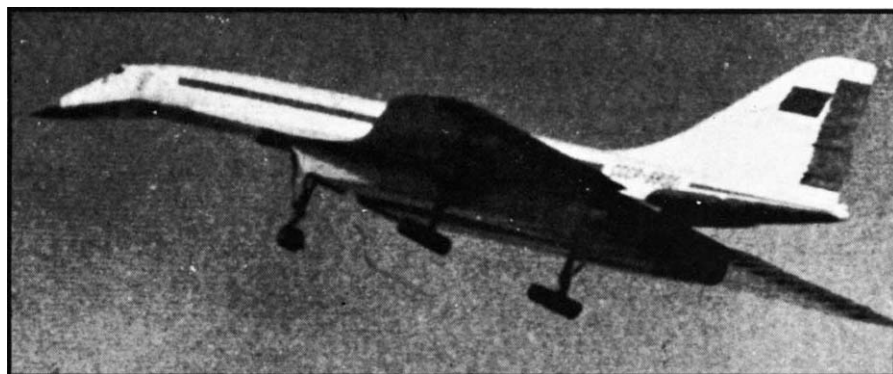
Given the possibility of such dire consequences resulting from the theft of comparatively tiny amounts of fissionable material, is the AEC making sure that the material is properly protected? Willrich and Taylor think not. For one thing, they point out that there are no specific requirements for the physical protection of less than 2 kilograms of plutonium, although that would be more than enough for a radiological weapon. And for another, they suggest that regulations governing communication with vehicles transporting fissionable material are not particularly effective.

But a safeguard system can be developed to keep the risk of theft to "very low levels". First, the study recom-

mends that the AEC should design a system of safeguards for each nuclear fuel cycle, based on the best technology available, and the agency should also consider the possibility of establishing a special federal security service whose sole job would be to protect nuclear material at fixed sites and during transit. Willrich and Taylor also suggested that careful consideration should be given to locating fuel reprocessing and fabrication plants side by side, to cut down the transportation of nuclear fuels between facilities. Finally, they suggest that the United States government should initiate discussions with other countries which have substantial nuclear power programmes in an effort

to develop a common policy towards international safeguards against nuclear theft anywhere in the world. The AEC's reaction is that its safeguards are more effective than Willrich and Taylor suggest.

But the credibility of the agency's safeguards received a severe blow last year when the General Accounting Office (GAO), a watchdog agency of the United States Congress, published the results of an inspection of three plants which handle strategically significant quantities of fissionable material. The GAO found evidence of laxity such as gaping holes in perimeter fences, inadequate burglar alarms and vulnerable storage facilities.



The TU-144: traumatic crash

Russians in the air from our Soviet Correspondent

AVIATION has always been considered a 'prestige' achievement in the Soviet Union, from the early 1920s when the propagandists of atheism solemnly argued that "There is no God for our brave airmen have failed to find him," through the spectacular air-lift, in March-April 1934, which evacuated 104 icewrecked survivors (including the inevitable newborn infant) from the polar research ship 'Semen Chelyuskin', through the 1945 anniversary celebrations of the Academy of Sciences when Joukowski received almost as much acclaim as Stalin up to the present day and the maiden flight of the TU-154 jet airliner from Moscow to Helsinki on April 9. The highly publicised crash of the TU-144 at last year's Paris air show, therefore, must have been a traumatic experience, not only for the Soviet aviation industry but for all concerned in the mass media.

According to the tenets of Socialist Realism, the press and other media, including the fine arts, should strive to present, not the transient defects of the world as it is but a sort of Platonic ideal of what it one day will be. The aftermath of the TU-144 disaster has, therefore, resulted in an even greater than usual press coverage of the positive aspects of aviation.

Nevertheless, since the Paris disaster there seems to have been a change in Soviet aviation policy, as reflected in

the press coverage. Whereas in February 1973, an *Izvestiya* article could state that the decision of PanAm and TWA not to take up options on Concorde was dictated not by commercial but by political motivation (since the United States "have nothing to compete with Concorde or the TU-144"), now the stress is all on co-operation with the United States. In January, a five-year cooperation agreement was signed between Lockheed and the USSR State Committee for Science and Technology, providing for the joint development of civil aircraft construction, navigation systems and aviation electronics. The inauguration of the direct Moscow-Washington air route was hailed by *Pravda* as "a major contribution to the further development in the extension of mutually advantageous economic cooperation of two great powers". For the moment, the negative aspects of competition seem to be minimised.

And yet since Concorde (in conjunction with the TU-144) has always been presented in the Soviet press as Europe's answer to the American aviation "threat", it is perhaps not without significance that, according to the Novosti agency, a factory at Kramatorsk (Ukrainian SSR) has begun work on a 60,000-tonne hydraulic press for the manufacture of large scale components of Concorde supersonic airliners.

OECD probing in Australia

Peter Pockley, Sydney

A TEAM of three examiners from the Organisation for Economic Cooperation and Development (OECD) is now in Australia for the first independent examination of the nation's organisation, funding and policies for science and technology. The team is led by Dr Alexander King, the OECD's Director-General for Scientific Affairs, who is an old hand at OECD science policy surveys; this will be his last one before retirement. Other members of the panel are Dr Freidrich Schneider of West Germany and Dr J. Wautrequin of Belgium.

Australia joined two dozen other nations as a member of the OECD just over two years ago and the current survey of Australia is the nation's first major examination under the organisation's critical microscope. The results should be interesting, not only for scientists but in a wider sphere as a measure of the nation's maturity in taking authoritative, international criticism on the chin and of the government's capacity for instigating sensible, perhaps radical, reforms which have not originated from internal, political pressures.

That such reforms may be recommended seems likely from the widely circulated comments of the OECD panel before its arrival in Australia at the end of March. Its comments were written in response to the background document prepared for the OECD by the Department of Science; they spoke of a good deal of thought being needed about weaknesses in the system, lack of balance and the need to develop in new directions. The OECD also noted the heavy concentration on government-run or government-sponsored science.

In a closely packed series of meetings, the team is now finding out for itself just how decisions of priority are made (or more to the point, how they are not made) in Australian science. Given the level of expenditure on research and development in Australia (now running at about \$A450 million a year), it may seem surprising that no similar survey has previously been conducted within Australia. Even now, there is no academic unit or independent institute in Australia which is professionally interested in the study of science policy on anything more than a part-time basis. The School of Sociology at the University of New South Wales is the nearest approximation to such a unit; senior staff are making a habit of spending sabbatical years in Britain at the Science Policy Research Unit at the University of Sussex.

Another continuing deficiency which the OECD team has already picked up, is the small amount of information in general circulation about the achievements and quality of scientific research *per se*. The background document presented by the government to the OECD confirmed this by dealing exclusively with the organisation of science in the country, almost as if the products of the research effort were hardly worth mentioning. The OECD examiners are hardly likely to be diverted from their stated objective of assessing the value of work done, as measured through accepted international criteria of scientific interest and merit. Their report should therefore prove doubly informative to Australians in positions of power who are ignorant of the very real achievements of science, notably outside the fairly well publicised government sector, such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

In the week before the OECD team arrived in Australia, the Minister for Science, Mr Bill Morrison tabled a Green Paper in the Parliament entitled *Towards an Australian Science Council*. Presented as a discussion paper rather than a blueprint, this document spells out the government's long-drawn-out uncertainty about the terms of reference and functions of the council, which they first proposed in their Labour Party Platform before the elections of December 1972.

In his speech about the green paper, Mr Morrison opened with a statement about "public disenchantment (which) has risen with the part played by science and technology in developments of dubious value to mankind". While this point can obviously not be ignored, the prominence given to it by the minister does cast doubts on his determination to bat vigorously in Cabinet on behalf of an expanding budget for science. All present indications are that funds for science will remain largely stagnant in relation to many other rapidly expanding areas of government spending in the country.

In this respect it is possible that the OECD examination is being quietly considered as providing justifications for different ways of slicing up the same financial cake. But, the OECD has made many acute observations in other countries, and it is equally possible that it will shake the assumptions of the Australian scientific bureaucracy and its political masters to the core.

Unfortunately, though, the effect of the report may be dulled within Australia by the present plan to present and discuss the report at the OECD headquarters in Paris, rather than in Australia.

European Earth scientists disunite

Peter J. Smith

ONE of the benefits often claimed for the recent revolution in the geological sciences is that it has finally succeeded in uniting solid-earth scientists of all disciplines. The new global tectonics, the argument goes, has provided a conceptual framework which enables almost every student of the Earth to find a place in a common enterprise. In the purely academic sense there may be some truth in it; but if recent events in Europe are anything to go by, interaction between geologists and geophysicists remains firmly bound between the covers of the learned journals.

The possibility of a serious breakdown in communication between the two groups first became apparent late last year when the General Secretary of the European Geophysical Society (EGS) discovered more or less accidentally that the Geological Society of London and the University of Reading were jointly planning a "meeting of European geological societies". For some time the precise purpose of the meeting remained unclear to the geophysicists, although it was widely believed that in addition to scientific sessions there would be an evaluation of the need and/or desire for the formation of a Geological Society of Europe (GSE). (One of the meeting's chief organisers said that no such thing was intended, although when the EGS General Secretary later relayed this information in good faith to a scientific conference in Peru it was flatly contradicted by another of the meeting's organisers).

Now that it has been formally announced that the meeting will take place at the University of Reading in September 1975, it is possible to see that its official object is "to provide an opportunity for members of European earth science societies to discuss (1) common scientific, economic and organisational problems, and (2) ways of promoting closer cooperation in future". The vagueness of this statement and the continued inability of the EGS General Secretary to get any more specific information from the sponsors of the Reading meeting have apparently upset many members of the EGS Council who feel that, whatever is going on, some prior consultation would have been both desirable and tactful. They are also concerned about the scientific theme of the proposed meeting, which is "Europe from crust to core". There being no such thing as the geology of the core, it is evident that the Reading meeting must incorporate some element of geophysics—

a subject which is already catered for on a Europe-wide basis by the EGS itself.

In spite of previous contradictory statements it seems likely that the possibility of a GSE will be discussed at Reading in some form. What particularly puzzles the EGS Council, therefore, is that the sponsors of the meeting apparently see no merit in drawing on the experience of the founders of the EGS and the council itself. After all, for several years prior to the formation of the EGS the problems involved in the cooperation of European earth scientists were discussed in great detail.

Given that European cooperation is desirable, the basic question as the geophysicists see it is whether there should be a single European Earth Science Society or whether geologists, geophysicists, geochemists and other such groups should go their own separate ways. Both arguments and individuals within the EGS seem to be about equally divided on this point. It is widely agreed that of overriding importance is the desirability of greater communication between earth scientists of all disciplines, a need increasingly dictated in any case by the way the subject itself is developing. On the other hand, if that implies the need for a single organisation, there are likely to be severe practical problems.

For one thing, geologists greatly outnumber the members of the smaller disciplines, and so there is a real danger that in an all-embracing body the latter group would become submerged—a thought which was very much in the minds of the founders of the EGS when the question of scope first arose. Second, there is the problem of logistics. More than 600 scientists attended the first EGS meeting held last year in Zurich; several thousands could be expected at a meeting of a combined European Earth Science Society. Quite apart from the fact that many people have expressed abhorrence at the mere idea of such a large gathering and that others have doubted the possibility of true communication in such conditions, suitable venues for very large meetings in Europe are surprisingly scarce. Moreover, one of the prime aims of the EGS in particular is to attract relatively impoverished students and research workers at the beginning of their careers; and this cannot be managed without cheap accommodation for conference participants. Finally, there is the point that there are still many geologists and geophysicists working in fields in which, rightly or wrongly, there is still little recognised need for cooperation with other branches of the Earth sciences.

The EGS believes that if a single European organisation with a single

annual meeting is to be ruled out, the best solution would involve individual bodies holding separate meetings at the same location but in successive weeks. In this way scientists could attend one, or the other, or both, of the meetings as they wish without doubling travel costs. The fact remains, however, that no satisfactory solution will emerge at all and chaos is likely to result unless geologists and geophysicists talk to each other. The EGS Council has now written to the organisers of the Reading meeting expressing concern at the meeting's scientific theme and formally requesting consultation on matters of mutual interest.

New union in Canada

from a Correspondent

ON February 22, 1974, the first meeting of a newly formed Canadian Geophysical Union (CGU) was held in Ottawa; it was chaired by the union's first president, Professor J. Tuzo Wilson of the University of Toronto. Canadian geophysics has been well served in the past by the National Research Council of Canada (NRCC) through its system of associate committees and sub-committees. Established in 1945 (also under the chairmanship of Professor Wilson) and active ever since, the Associate Committee on Geodesy and Geophysics has been the main co-ordinating body, the clearing house, for scientific and technical information, and the benevolent patron of most geophysical research in Canada. The main activities of the Associate Committee were semiannual meetings at which the progress of various research projects were discussed and early scientific results announced, and the publication of an annual *Geophysical Bulletin* containing the reports of activities of all important centres of geophysical research in Canada. Twenty-five published volumes of this bulletin provide an opportunity for all geophysicists to find out about programmes of research outside their own field. But the NRCC itself is being reorganised and as part of this process geophysicists have been nudged out of their warm and cosy nest.

The programme of the inaugural meeting consisted of two parts: a presentation of "Frontiers of Science" lectures and a panel discussion on the future of geoscience in Canada. The first part suggested that the progress of geophysical research is healthy; the second part suggested that the assembled membership did not anticipate any serious problems ahead. Surprisingly, there was no mention of shortages of research funds although there were polite suggestions that more effort

should be devoted to this or that project (theoretical geophysics, geodynamics of the Earth's interior, exploration geophysics research and so on). The question of whether the participation of Canadian geophysicists in international science is at an adequate level was discussed but no conclusions were reached. Also unanswered was the question: "What are the government organisations responsible for regional geophysical surveys going to do when these regional surveys have been completed?"

The CGU will be the national body adhering to the International Union of Geodesy and Geophysics (IUGG). Its stature at the international level will be assured by the support it has from prominent Canadian geophysicists. The strength of its voice in national affairs will depend on the membership it can attract and there will be competition with a number of specialised societies loosely federated in the Canadian Geoscience Council. Because of the size and the geographical situation of the Canadian land mass and the adjacent offshore areas, geophysicists working there have always taken their responsibilities to international science seriously. On the continental scale these responsibilities have included standardised seismological networks, regional gravity maps, surveys of the magnetic pole and research on Aurora. On the scale of global geophysics the new union has an important role to play and the international scientific community will watch its growth with interest.

Medical research in the dock

A Boston grand jury has set the stage for two separate court battles, one of which holds important implications for medical research, and the other involves a legal challenge to a key aspect of the historic decision on abortion which was handed down last year by the US Supreme Court.

In the first case, four medical researchers have been accused by the Grand Jury of violating an obscure Massachusetts law in connection with a research project they carried out at Boston City Hospital in 1971 and 1972. If the accusation stands up in court, it could put an end to all research on foetal tissue in Massachusetts.

The research involved giving antibiotics to 33 women scheduled to undergo therapeutic abortions, in an attempt to determine which of the drugs was more effective in crossing the placenta. The objective was to find an antibiotic to use instead of penicillin for curing foetal infections. After the abortions were performed, the dead foetuses were

analysed for signs of drug residues.

The doctors have been charged with illegal dissection of non-living tissue under a 19th century Massachusetts law designed to prevent graverobbing. Medical researchers in the Boston area have been quick to point out that research on dead foetal tissue has played an important part in the development of vaccines and that foetal tissue is often vital for all sorts of medical investigations. In fact, two Harvard scientists, Dr Thomas Weller and Dr John Enders, won the Nobel Prize in 1954 for growing polio virus in cells cultured from foetal tissue.

If the prosecution is successful, the ban on such research would extend only to Massachusetts, but since there is a huge medical research complex in the Boston area, its effect would be very keenly felt. In the meantime, the Massachusetts State Legislature is considering a bill which would impose a flat ban on research involving aborted foetuses, so even if the ancient graverobbing law proves to be ineffective, Boston scientists are still in danger of being saddled with legal prohibitions on their work.

The second case involves an indictment against Dr Keith Edelin, Chief Resident in Obstetrics and Gynaecology at Boston City Hospital, who has been accused by the same Grand Jury of manslaughter in connection with a legal therapeutic abortion which he performed last October. He has been charged with killing a foetus, reported to be between 16 and 24 weeks old, which the prosecutor's office maintains was a viable human being.

When the case is eventually brought to court, the central issue will revolve around the responsibility of a doctor to do all in his power to keep an aborted foetus alive. This is an issue which was left rather murky by the Supreme Court, which argued that the state has a right to protect the life of an unborn child only when it is 'potentially able to live outside the mother's womb, albeit with artificial aid'. This usually occurs, the court said, at 'about seven months'.

The Supreme Court decision overturned a Massachusetts anti-abortion law, and Edelin's indictment has been viewed by some observers as an attempt to challenge the court's decision and to define more rigidly the concept of viability. In Catholic Boston, there is a very potent anti-abortion lobby.

Edelin's colleagues at Boston City Hospital have published a statement suggesting, in fact, that he may be a scapegoat for the anti-abortion forces, and they have predicted that he will be cleared when the facts are known. In any case, the court case is going to be closely watched throughout the country.

news and views

Water, myth and magnetism

IN these days of the Geller effect, the perhaps magnificently lost causes of quarks and gravity waves and the great international *Bockschiessen* over polywater, it would be a pity to lose sight of the fact that, at any one time, there are probably a handful of minor absurdities lurking on the fringes of orthodox physics which, although lacking some of the sensational character of the examples mentioned, seem nonetheless worth bringing into the open.

One such case recently drew me into previously unsuspected backwaters of the physicochemical literature to a number of articles which, though preposterous in many respects, seemed to leave enough important questions unanswered to be worth describing. It concerns the alleged possibility of producing mysterious changes in the physical properties of water by the application of relatively weak magnetic fields—permanent changes or transient ones depending which of several authors one is inclined to believe.

The subject of the magnetism of water surfaced briefly in the mainstream literature about two years ago with a perfectly unsensational paper by Quickenden, Betts, Cole and Noble of the University of Queensland (*J. phys. Chem.*, **75**, 2830; 1971) who carried out a meticulous null experiment showing, to nobody's great surprise, that no detectable changes in pH can be found in magnetically treated water, none being in any case remotely conceivable in terms of any electronic model for the water molecule. It is in the more obscure papers which inspired Quickenden *et al.* to undertake such a seemingly futile measurement that the case becomes curiously and curiously.

Their immediate stimulus was a paper by Joshi and Kamat (*J. Indian chem. Soc.*, **43**, 620; 1966) claiming to have obtained not only changes in pH of about half a unit in CO₂-equilibrated water but—for good measure—distinct, irreversible changes in surface tension and dielectric constant as well, all with fields little stronger than that of a permanent bar magnet. At this point the armchair theorist (or rather one perched high on a Patent Office stool) finds that the subject extends back from familiar territory into the somewhat alien world of hydrology, boiler-feed management and the suppression of incrustations in the conduits of spas and watering places. It seems that, for a considerable time, boiler engineers in many parts of the world have been passing their feed water through quite mild magnetic fields and obtaining a sensational reduction in the deposition or, more correctly, the adhesion of scale as a result. A wave of testimony to this passed through the relevant literature about ten years ago, with rapturous accounts originating as far apart as the Works for Heavy Machinery Construction in Alma Ata (Bitny *et al.*, *Bezop. Truda v Prom.*, **3**, 22; 1959) and the Belgian Institute for Corrosion Research CEBELCOR in Brussels. Some accounts claim that passage of feed water through the field of a small bar magnet can actually lead to removal of scale already deposited, patents have been taken out and there is mention of a figure of 50,000 successful installations of the "CEPI" device, embodying a small permanent magnet in the inlet.

When I took this up with one particularly wise and ex-

perienced physical chemist, his reaction was that this was nothing compared with some of the almost magical practices resorted to by boiler engineers. For instance, had I heard of the use of glass globes containing helium and a little mercury tethered in the water supply and said to be almost as effective as the magnetic system? I had not but some further research turned this up too, in an article by Friedel (*Chem. Ztg.*, **84**, 539; 1960) who attempts to explain the electrochemistry of both this, the "Tonisator" as it is called, and the CEPI method. Friedel's explanations, invoking Geissler discharges and a permanent magnetically induced strain in the bond angles of the H₃O⁺ ion, are far fetched by any account, though this might be held to be of little concern to the improbable 50,000 maintenance men the world over, released by the CEPI device from the unpleasant task of periodic chipping out with a descaling hammer.

But, if one must single out the most remarkable paper of all on water magnetism, it must surely be that of Bruns, Klassen and Kon'shina of the A. A. Skochinskii Mining Institute in Moscow (*Colloid J. USSR*, **28**, 129; 1966), likewise backed up by another whole subliterature from Russian specialist sources. Bruns and colleagues go further than the other work cited in two respects. They claim that the optical absorption of water can be altered up to some 30% by quite moderate magnetic fields, the treated water regaining its normal state with a time constant of some hours, and further that the effect is periodic in the applied magnetic field strength. (Here, however, it must be solemnly recorded that some of the 'periodic' data presented is drawn with two maxima and minima spaced among only five data points.) The explanation proposed is that there is a 'resonance' interaction with the vibratory motion of groups of associated water molecules under the influence of the field . . .

After all this it seems almost hubristic to suggest that, if magnetic fields can really interfere with the pH or any other electrochemical property of water significantly, it might be a very painful and hazardous business putting one's finger between the poles of a strong electromagnet. (True that blood is effectively buffered, but why should the buffer pKs be any more immune to magnetic interactions?) Of course biological effects of magnetic fields are known but they are weak and elusive and, on the whole, poorly documented. Mice show no particular ill effects from a few hours at 150,000 oersted and men have been exposed to 20,000 oersted for a quarter of an hour or so. Meanwhile, at the invertebrate and cellular level, there is much dispute as to whether fields can cause abnormalities in *Drosophila*, sea urchin eggs, photobacteria and ascites tumour cells though certain creatures such as the mud snail *Nassarius obsoletus* seem candidates for strange susceptibilities. (For details of these experiments and much else see *Biological Effects of Magnetic Fields* (edit. by Barnothy, M. F.), Plenum, New York, 1964). Of the various marginal effects, magneto-tropism in plants seems the best established. From these matters it is but a short step to the very strange phenomena of dowsers and bird navigation and their attendant literature, as rich in speculation as short on physical theory. Hall effects and π -electron interactions in proteins, perhaps, but only by wildest stretch of the imagination (and the wild stretching of imagination is common enough in such fields) attribution to physical changes in water.

So what in the end of the contented boilerman in Alma Ata and the guidance system of the mud snail? Can these

conceivably have enough in common to warrant a new, close look at the magnetophysics of aqueous systems—or should physical chemistry, with uneasy glances over the shoulder at the polywater nonsense, let this particular sleeping dog lie? For it could well be that all the various effects have but one essential in common, their ability to elicit man's tendency, active since ancient times, to relate the mystery of the magnetic force and its field to almost every other enigma that troubles him. A tendency to which a remarkable variety of scientists seem in no way immune.

From our Molecular Physics Correspondent

Continuity of Precambrian life

ALTHOUGH Precambrian microfossils have been known for many years, little attention was given to this area of investigation until after the descriptions of the microorganisms from the 1.9×10^9 year old Gunflint Chert^{1,2}, part of the Lake Superior banded iron formations from Canada. These publications appeared in 1965 and initiated the examination of many ancient rocks by scientists of various disciplines in an endeavour to throw light on the origin and evolution of living systems. This great burst of interest coincided with the scientific activities associated with the preparation for the American manned space flights and lunar landings which renewed interest in the possibilities of extraterrestrial life, its origin and possible contamination of the Earth by returning astronauts.

The problem was tackled on a broad front. The chemists investigated both the soluble and insoluble fractions of the organic matter in Earth rocks, meteorites and, later, lunar rocks. The palaeontologists examined the rocks for evidence of morphologically preserved microfossils, and found these in Earth rocks. There have never been any indications in lunar rocks of structures possibly interpretable as biogenic in origin and the bizarre, chemically organic, six-sided box-like structures from the carbonaceous chondrites have been interpreted as condensation products of abiogenically produced organic compounds³. The small hollow, spherical organic bodies also recovered from carbonaceous chondrites have been similarly interpreted⁴, and it is significant that the surface texture of the meteorite spheroids is markedly different from the surface texture of spheroids from the 3.355×10^9 yr old Onverwacht Group rocks of South Africa^{4,5}.

A survey of the earlier work can be found in Schopf⁶. Three time intervals have received considerable attention: $3.4-3.0 \times 10^9$ yr; around 2.0×10^9 yr; and from 1.3×10^9 yr to the base of the Cambrian.

In the first of these time intervals occurs the oldest, well dated, easily accessible sedimentary succession, the Onverwacht Group of the Swaziland System of South Africa. The 3.750×10^9 yr old Amitsook and Issua rocks of Greenland are less accessible, and in the case of the Amitsook, highly metamorphosed. It would be interesting to see the results of the $\delta^{13}\text{C}$ analysis on the graphite from these rocks, although the high grade of metamorphism would make interpretation of the results very difficult.

Even in the Onverwacht Group, the rocks are part of a green stone belt, they have been folded and have suffered some degree of metamorphism⁵. Nonetheless, small (up to 20μ) spheres and filaments have been found in moderate abundance in the cherts, and their biogenicity has in general been supported by the evidence of the $\delta^{13}\text{C}$ ratios of the kerogen⁷. The kerogen itself has a chemical structure similar to that of kerogens produced by degradation of known biogenic compounds⁵. Controversy centres on the older part of the Onverwacht, the Theespruit Formation. The $\delta^{13}\text{C}$ ratios of its organic matter are anomalously low

and this may imply the presence of possibly pre-biogenic or at least pre-photosynthetic vital activity. This difficulty cannot be resolved however, until the effects of metamorphism by heating and by pressure on $\delta^{13}\text{C}$ ratios are better understood.

Evidence for early photosynthesis is adduced from the discovery of stromatolites in the Rhodesian 3.1×10^9 yr old Bulawayan System⁸, that is roughly the same age as the Fig Tree Group which directly overlies the Onverwacht Group of South Africa. Microfossils in the Fig Tree Group were first discovered and named by Barghoorn and Schopf¹⁰. Although some doubt has been cast on the validity of the rod-like 'bacterial' forms discovered only by electron microscopy⁶, the spheres discovered in optical thin section are regarded as reliably biogenic⁷ and further investigation of these rocks is necessary.

Until very recently, a huge gap in knowledge existed between the Fig Tree spheroids and the banded iron formation microorganisms—a time gap of nearly 1,000 Myr—but two groups have recently described^{11,12} the discovery of extremely well preserved blue green algae in dolomitic limestones of the 2.3×10^9 yr old stromatolitic Transvaal System. These algae have a remarkably modern aspect and have been closely compared with modern genera of blue green algae. These fossil assemblages are unusual in that they are found in dolomitic limestones and not in chert.

The banded iron formation Gunflint Chert assemblage^{1,2} is associated with stromatolites and is remarkably varied and modern in appearance. Walter¹³ has suggested that these may represent fossilised fumarolic hot springs deposits and that they may have been preserved in primary silica resulting from volcanic activity. The microfossils from other banded iron formations, in Australia for example, do not however, resemble the Gunflint organisms at all, and indeed are much more similar to the spheres from the much older Onverwacht and Fig Tree rocks, both in appearance and in the state of organic metamorphism. The differences may result from original facies variations, or from the very slight age difference between the Australian (2.0×10^9 yr) and the Canadian (1.9×10^9 yr) rocks.

Until fairly recently, there was nearly as large a gap in knowledge between the Gunflint assemblages and the Bitter Springs assemblages described by Schopf^{6,7} as between the Gunflint and the Fig Tree. The Bitter Springs assemblage contains many unicellular and multicellular blue green algae, and has a moderately high proportion (about 25%) of cells interpreted as eukaryotic from the presence of preserved organelles and from the type of cell division observed.

Similar cells, containing membrane-bound contents have since been reported from Beck Springs, California (1.4×10^9 yr old)¹⁴, Belcher Island, Canada (1.8×10^9 yr old)¹⁵ and, most recently, from the Bungle Bungle Dolomite, Western Australia (1.5×10^9 yr old)¹⁶. Thus the origin of the eukaryotes must be regarded as having occurred much earlier than has previously been supposed⁷. The Bungle Bungle Dolomite contains many colonial and multicellular microorganisms like the recently reported assemblage from the Amelia Dolomite of the McArthur Group of Australia's Northern Territory, also 1.6×10^9 yr old^{17,18}. Colonial microorganisms have also been recovered from the HYC ore body of the McArthur Group¹⁹, lending strength to the hypothesis that deposition of some stratiform ore bodies may be related to biogenic activity. This same view has been put forward by Lopukhin²⁰, in a review of Soviet Precambrian microorganisms and their economic importance.

Although there now seems to be some evidence for the presence of eukaryotic organisms as long ago as 1.8×10^9 yr, the assemblages from $1.8-1.3 \times 10^9$ yr interval, although very varied, are dominated by prokaryotes^{16-18, 21}. The cells described by Hoffman²¹ from Belcher Island may well represent primitive 'protoeukaryotes', as indeed may

the cells described from the older Animikie Formation of Ontario²².

New assemblages have been described from the later Precambrian^{23, 24}, and these contain cells that can reasonably be interpreted as eukaryotes thus reinforcing some of Schopf's⁶ earlier views. The enigmatic *Chuarina*, with a diameter of between 2 and 3 mm, has been redescribed by Ford and Breed²⁵, and assigned to the Leiospheridia (Acrutarcha). All the recorded occurrences of *Chuarina* are from Precambrian rocks, usually shales, less than 1.0×10^9 yr old. The occurrence of these large plant microfossils seems to have stratigraphic importance. Furthermore, the Leiospheridia can be related to the present day eukaryotic Prasinophyceae.

By the very late Precambrian, complex green algae, similar to *Botryococcus* have been observed in the lower part of the Postspilitic Group from Vrané, near Prague²⁶.

These recent discoveries tend to modify earlier views⁶ that "gradually accelerating early evolutionary development, beginning rather slowly but markedly quickening with the emergence of eukaryotic organisation, seems consistent with the fragmentary evidence currently available". A model of a more gradual, steady, development seems to fit the present evidence much better. Furthermore, the new evidence includes the discovery of microfossils in lithologies other than the facies-restricted black, usually stromatolitic cherts of earlier years, and these will undoubtedly greatly enlarge knowledge of Precambrian microbiotas. M.M.

¹ Barghoorn, E. S., and Tyler, S. A., *Science*, **147**, 563 (1965).

² Cloud, jun., P. E., *Science*, **148**, 27 (1965).

³ Brooks, J., and Muir, M. D., *Grana*, **11**, 9 (1971).

⁴ Rossingol-Strick, M., and Barghoorn, E. S., *Space Life Sci.*, **3**, 89 (1971).

⁵ Brooks, J., Muir, M. D., and Shaw, G., *Nature*, **244**, 215 (1973).

⁶ Schopf, J. W., *Biol. Rev.*, **45**, 319 (1970).

⁷ Schopf, J. W., *Origins of Life*, **5**, 119 (1974).

⁸ Oehler, J. D., Schopf, J. W., and Kvenvolden, K. A., *Science*, **175**, 1246 (1972).

⁹ Schopf, J. W., Oehler, D. Z., Horodyski, R. J., and Kvenvolden, K. A., *J. Palaeontol.*, **45**, 477 (1971).

¹⁰ Barghoorn, E. S., and Schopf, J. W., *Science*, **152**, 758 (1966).

¹¹ Nagy, L., *Science*, **183**, 514 (1974).

¹² MacGregor, I. M., Truswell, J. F., and Eriksson, K. A., *Nature*, **247**, 538 (1974).

¹³ Walter, M. R., *Econ. Geol.*, **67**, 965 (1972).

¹⁴ Cloud, P. E., jun., Licari, G. R., Wright, L. A., and Troxel, B. W., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 779 (1968).

¹⁵ Hofmann, H. J., and Jackson, G. D., *Can. J. Earth Sci.*, **6**, 1137 (1969).

¹⁶ Diver, W. L., *Nature*, **247**, 361 (1974).

¹⁷ Croxford, N. J. W., Janecik, J., Muir, M. D. and Plumb, K. A., *Nature*, **245**, 28 (1973).

¹⁸ Muir, M. D., *Origins of Life*, **5**, 105 (1974).

¹⁹ Hamilton, L. H., and Muir, M. D., *Mineralium Deposita* (in the press).

²⁰ Lopukhin, A. S., *Geol. För. Stockh. Förh.*, **95**, 329 (1973).

²¹ Hofmann, H. J., *Nature*, **248** (in the press).

²² Edhorn, A.-S., *Geol. Assoc. Can.*, **25**, 37 (1973).

²³ Schopf, J. W., and Fairchild, T. R., *Nature*, **242**, 537 (1973).

²⁴ Schopf, J. W., Ford, T. D., and Breed, W. J., *Science*, **179**, 1319 (1973).

²⁵ Ford, T. D., and Breed, W. J., *Palaeontology*, **10**, 535 (1973).

²⁶ Konzalova, M., *Vest. ústred. Úst. Geol.*, **48**, 31 (1973).

In that other 5% or less, the limb may still be conjured up by concentrating on it, or by trying to move it.

In most limb amputations, the cut nerves end up in firm swellings called neuromata which consist of whorls of outgrowing nerve fibres surrounded and intertwined by connective tissue. One assumes that these nerve fibres are capable of working and that they frequently fire off impulses to the central nervous system. Not only is this a likely assumption, but it is reinforced by the effect of blocking the cut nerves of the neuroma with a local anaesthetic. When these nerve fibres are blocked in this way, there is a great reduction of sensation or a total loss of the sensation of the phantom limb. It is apparent that the sensation was due to the nerve fibres within the neuroma continually firing off impulses.

A few people who have had parts of their bodies amputated, experience constant pain in the stump or in the phantom, or both. In such patients, blocking the neuromata with local anaesthetics almost always temporarily stops the pain. Many suggestions have been proffered as to why some of the patients have pain and others do not. It could be caused by a difference in the make-up of the neuromata, but this is an unsatisfactory suggestion because no differences in the histological appearances between pain producing and painless neuromata have been found.

Wall and Gutnick (see this issue of *Nature*) have now induced neuromata in the cut sciatic nerves of rats and have recorded from the posterior rootlets entering the spinal cord. As was expected, they found that the nerve fibres of these neuromata were continually active. Their discharge of impulses could be increased by pressure or tapping on the neuromata and it could be stopped by anaesthetising the neuromata with local anaesthetic solution—all similar to the situation in man.

A more interesting finding was the effect of electrical stimulation of the chronically divided nerve. The nerve on which the neuroma had been induced was divided so that it no longer reached the spinal cord. Electrodes were then placed on the nerve and it was stimulated rapidly for a few seconds. This electrical excitation caused a marked reduction in the nerve's spontaneous activity, which lasted for up to an hour. This finding may suggest that if the nerve fibres can be induced to fire off maximally, they may stop firing spontaneously for a prolonged period. A similar mechanism could account for the effectiveness of one of the treatments for painful neuromata. In 1881, Granville (*Lancet*, i, 286) introduced percussion of the neuroma as a treatment for the chronic pain. This treatment was largely forgotten, revived for a short time during the 1914-18 war, forgotten again, and re-introduced by Russell (*Br. med. J.*, **1**, 1024; 1949) the results being reported by Russell and Spalding in 1956 (*Br. med. J.*, **2**, 68). Sometimes when the patient first hits his neuroma with a mallet and peg, the pain is increased; but the neuroma soon becomes numbed and the pain may then stay away for hours.

During the past few years the transcutaneous electric stimulation of nerves has been introduced for many painful conditions, including painful amputation stumps and painful phantom limbs. This treatment was based on the gate-control theory of pain of Melzack and Wall (*Science*, **150**, 971; 1965). The theory states that whether or not an input to the spinal cord finally causes pain depends on the amount of input arriving in large myelinated afferent fibres on the one hand and small myelinated and non-myelinated afferent fibres on the other. Both groups of fibres are said to affect the small neurones of the substantia gelatinosa, a part of the posterior horn very near the zone of entrance of the afferent fibres. The large fibres excite these neurones and the small fibres inhibit them. These substantia gelatinosa neurones are inhibitory. And so, when these neurones are themselves inhibited, their inhibitory action on the afferent fibres will not be exerted; a massive afferent input will enter

Help for the painful phantom limb?

THE phantom limb is a phenomenon that has long aroused curiosity. How is it that one feels a limb that is not there? Yet once one thinks about the anatomy and physiology of the situation, one realises that phantom limbs are to be expected: all the nerves coming from the limb are still there, they still make connections at various levels of the central nervous system and, finally, these connections connect to that part of the brain where afferent nerve impulses evoke sensation. Phantom limbs occur in 95 to 100% of all people who have had a limb or part of a limb amputated.

the spinal cord, and pain will result. When these neurones are excited, they exert presynaptic inhibition on afferent fibres, and the input to the spinal cord will be moderate; pain will not occur. Excitation of the large afferent fibres thus reduces the input to the spinal cord and is said to close the gate.

Wall and Gutnick now suggest an alternative way in which electric stimulation might act. It could do so preventing nerve impulses being fired off spontaneously, and, as it is this firing that causes both the pain and the phantom sensation, both would be stopped by electric stimulation. The mechanism by which antidromic stimulation stops impulse generation after stimulation has stopped its unknown. Wall and Gutnick suggest that the excitability of small nerve fibres within the neuroma is altered by electric stimulation because these endings are abnormal and are not behaving in the same way as do normal nerve fibres; and they bring forward some evidence to show that this is so.

This is probably the first physiological investigation to have been carried out on a traumatic neuroma induced in an animal. It seems as if it has already thrown light on how certain forms of therapy may work; and it suggests that a similar investigation could be carried out in man, now that it is possible to record from human nerves by means of electrodes introduced into the peripheral nerves. P.W.N.

Unpredictability of comets

from a Correspondent

COMET Kohoutek disappointed many people over Christmas by being considerably fainter than predicted. Most of the blame for this disappointment can be laid at the feet of the media-men who seem to hanker after superlatives such as brightest, longest, most spectacular and are not interested in run of the mill comets and the qualified predictions of astronomers. Comets themselves, however, must also carry some of the blame as they are notoriously unpredictable. This cometary fickleness has been stressed in an article by Jacchia, who works at the Center for Astrophysics, Harvard Observatory and Smithsonian Astrophysical Observatory (*Sky Telesc.*, 47, 216; 1974).

The brightness J of a comet can be represented by the formula $J = J_0 \Delta^{-2} r^{-n}$ where J_0 is a constant, Δ is the distance between the comet and the Earth, r is the distance between the comet and the Sun, and n , the power of r , is a quantity that varies from comet to comet and in certain cases as a function of r .

In an extensive study by Bobrov-

nikoff the mean value of n was found to be 3.3. This indicates that cometary luminosity is produced by two mechanisms; first, the reflection of sunlight from the small dust particles in the coma (if this was the only source n would be 2) and, second, by direct emission from gas molecules, thus producing band spectra. The luminosity increases as the comet approaches the Sun because of the increase in the incident radiation, coma size, gas and dust production and molecular excitation. Increases in luminosity and therefore brightness, however, do not always occur and n has been known to take values that range from -1.77 to $+11.40$. This variability in the power of r is indicative of the chance one takes when predicting cometary brightness. Jacchia gives as one telling example the case of comet Westphal which was observed as an 8th magnitude object on September 26, 1913. Perihelion passage was due on November 26 but instead of brightening the comet gradually faded being 13 mag at the end of October and 17 mag on November 22, 4 days short of perihelion, when it had been expected to be at its brightest.

Jacchia compares Kohoutek with comet Halley's apparition in 1910. Halley performed excellently having an n of 5 and also brightening in the post-perihelion phase. Kohoutek started well with an n of 4.0 shortly after discovery (justifying the early optimistic predictions) but n dropped to 3.0 in early November 1973 and 2.2 around December 20. At perihelion the comet shed more matter than expected and was easily photographed by the Skylab astronauts, but after perihelion the comet followed a trend one magnitude fainter than it did at the corresponding distance on the way to the Sun—exactly opposite to the behaviour of Halley's comet.

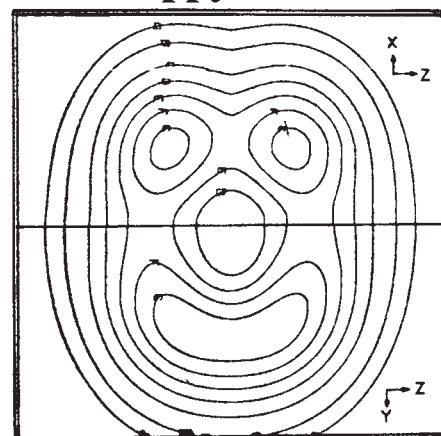
So comets seem to have two records in astronomy. Not only do they have the greatest range of brightness of all celestial objects, the great comet of 1882 being seen in full daylight 4 degrees away from the Sun and comet Wolf 1 being observed in 1942 when it had a magnitude of 19.3, but they also seem to be the most unpredictable: comet Kohoutek unfortunately lived up to this latter reputation.

Man misled by man—on ice

from our Geomagnetism Correspondent

THERE is a standing joke among palaeomagnetists about the man who spent several weeks collecting beautiful samples of baked red laterite. When he returned to the laboratory one of his more experienced colleagues coolly

Happy nuclei



Hartree-Fock calculations of nuclear matter have now advanced to the stage where they can reveal quite detailed and unexpected features of nuclear structure. This illustration shows the contour plot of the density of sulphur-32 obtained by Curry and Sprung (*Nucl. Phys.*, A216, 125; 1973).

informed him that he had drilled the brick foundations of a long-demolished farmhouse. Each branch of the earth sciences has its own variant of this story, but for glaciologists the joke seems to have come uncomfortably to life. As the recent experience of two North American scientists demonstrates, it is easy to be misled by one's ancestors even in the most unlikely of places.

Last year, Berkland and Raymond (*Science*, 181, 651; 1973) reported that they had found glacial polish, grooves and striations at a height of 1,370 m on Grandfather Mountain in North Carolina. The longest of the grooves were about 1 m long, 15 cm wide and 5 cm deep, and the 60 or so recognised examples gradually changed their trend from S80°E to S40°E from the southern to the northern ends of the exposure. This variation, Berkland and Raymond argued, indicated "two lobes of ice which formerly coalesced at the site of the outcrop and preserved it as a bastion". They then went on to calculate that the ice had maximum and minimum thicknesses of 300 m and 100 m, respectively, in the cirque containing the outcrops investigated. It is true that no moraines were observed at the cirque outlet, but this was considered neither unusual nor significant.

What was considered significant was that Grandfather Mountain lies at a latitude of about 36°N, whereas alpine glaciation in the eastern United States had not previously been reported south of the Catskill Mountains in New York state (about 42°N) and the Laurentide ice sheet ended in New Jersey and Pennsylvania between 40° and 41° N. In other words the new result suggested that Pleistocene glaciation had extended some 890 km further south in the

Appalachians than had previously been supposed. This, in turn, raised questions about other evidence which had hitherto been interpreted differently. Striated boulders and cobbles, for example, are quite abundant locally in Pleistocene river gravels of North Carolina and Tennessee and are indistinguishable from glacially striated clasts. Wentworth (*Bull. geol. Soc. Amer.*, **39**, 941; 1928) suggested the possibility of a glacial origin for these more than 40 years ago, although both he and later workers have preferred to ascribe their markings to river ice. The new discovery made by Berkland and Raymond, if substantiated, suggested that the question of glaciation in the southern Appalachians would need to be re-examined.

Unfortunately, the discovery has not been substantiated; that is to say, although there is no doubt that the grooves exist, their origin is certainly not glacial. McKeon (*Science*, **184**, 88; 1974) now reports that he has examined the grooves and has found, among other things, ferric stains in the bottom of some of them, polish on groove bottoms but not on intergroove surfaces, the absence of grooves in shallow lows of the outcrop surface, and the presence of at least one groove continuing part of the way down the outcrop's vertical face. These characteristics, he argues, suggest that the grooves are not glacial but man-made. More specifically, he suggests that the grooves are a product of stone quarrying, logging or survey activities during the past two centuries—a suggestion strongly supported by the discovery of weathered wire cables downslope from the outcrop. McKeon also uses data from a weather station on Grandfather Mountain to show that glaciation at an elevation of 1,370 m would imply a July glacial age temperature about 18° C lower than the present July temperature—a decrease three times greater than that usually acknowledged for the ancient glacial areas.

Hack and Newell (*Science*, **184**, 89; 1974) have also visited the outcrop concerned and confirm the characteristics described by McKeon, adding that the grooves "in no way resemble the polished, striated, and grooved outcrops [they] have seen in glaciated areas". They also found the rusty steel cables, but were more enterprising than McKeon in seeking out a retired lumberman who had taken part in the last logging in the area during the 1930s. This gentleman described precisely how the grooves were formed by the operation of a steam-powered cable system, adding that similar grooves could be found "on rocks all through the woods". In short, it is clear that the grooves found by Berkland and Raymond are of more interest to industrial archaeologists than to glaciologists.

To their credit, Berkland and Raymond (*Science*, **184**, 89; 1974) openly admit that they were mistaken about the origin of the grooves, at the same time describing at length how they came to be misled. On the other hand, they disagree strongly with Hack and Newell's statement that "no need exists for philosophical discussion of the possibility of alpine glaciation" in the southern Appalachians. On the contrary, they assert that there is a wealth of physical, biological and palaeoclimatic evidence to support such glaciation, and promise that Berkland will discuss this more fully in a later publication. It is clear that, in spite of the set-back of the misinterpreted grooves, the issue of southern Appalachian glaciation is still far from dead.

Palindromes in eukaryotic DNA

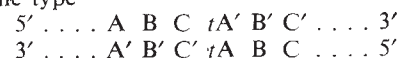
from a Correspondent

GONE are the days when genomes were described solely in terms of the abundance of different repetition frequency classes in eukaryotic DNA sequences. Nowadays it is fashionable to write of the interspersed of repeated and non-repeated sequences and to formulate models of the genome based on the lengths of repeated and non-repeated elements and the extent of intermingling. Good evidence has been provided in *Xenopus* (Davidson *et al.*, *J. molec. Biol.*, **77**, 1; 1973), sea urchins (Graham *et al.*, *Cell*, **1**, 127; 1974), *Drosophila* (Wu *et al.*, *J. molec. Biol.*, **64**, 211; 1972; Lee and Thomas, *ibid.*, **77**, 25; 1973, *Necturus* and mouse (Pieritz and Thomas, *J. molec. Biol.*, **77**, 57; 1973) for such a model. Most of this work, however, has dealt specifically with the intermingling of intermediate repetitive with non-repetitive sequences and not with the fast renaturing, highly repetitive, low complexity DNA, such as mouse satellite DNA, which is thought not to contain any single copy elements over large stretches. The integration of DNA sequences of this type, known to be predominantly in centromeric DNA by *in situ* hybridisation, may be in large blocks alternating with sequences of greater complexity. Evidence in favour of this idea has come from equilibrium caesium chloride density gradient studies on the integration of low complexity satellite DNA sequences in the *Drosophila* genome (Kram *et al.*, *J. molec. Biol.*, **64**, 103; 1972).

There is a fourth fraction of eukaryotic DNA which renatures spontaneously at zero time even at very low DNA concentrations and which has received little attention until recently. Davidson *et al.*, in their paper on interspersed of repetitive and non-repetitive sequences in *Xenopus* also

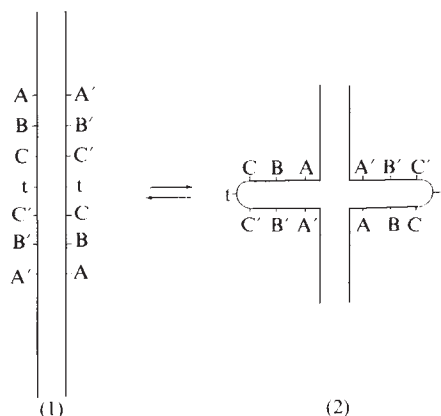
found that this zero time renaturation increases with fragment size and suggest that there is a relatively small fraction of single stranded DNA molecules containing tandem self complementary sequences capable of forming intra-strand duplexes.

Wilson and Thomas (*J. molec. Biol.*, **84**, 115; 1974) now provide further evidence for the existence in eukaryotic DNA of inverted repeated sequences of the type



called palindromes by analogy with words and phrases which read the same backwards as forwards. Denatured palindromes would be expected to renature by an intramolecular reaction to form hairpin loops. The rate of hairpin formation is predicted to be very swift, depending on the number of nucleotides (t) separating the inversion, and should proceed at very low DNA concentrations since complementary sequences will not be diluted.

What Wilson and Thomas have done is to use hydroxyapatite, which binds double stranded but not single stranded DNA at low salt concentrations, to prepare spontaneously renaturing DNA from a variety of sources (HeLa cells, mouse L cells, *Triturus* liver cells, and *Drosophila* flies). They show that zero time binding is independent of DNA concentration and occurs at C_0t values (10^{-5}) of the same order as the $C_0t_{1/2}$ for the reaction between poly(dA) and poly(dT). The only variable which seems to influence the amount of zero time binding is the molecular weight of the original DNA. It increases linearly with molecular weight except in *Triturus* where a plateau is reached with 60-70% DNA bound at molecular weights greater than 10^7 daltons. In HeLa DNA the maximum amount bound increased up to 80% at a molecular weight of 6×10^7 daltons.



(1) Linear palindrome; (2) cruciform palindrome (after Wilson and Thomas).

An alternative explanation that zero time binding is due to the presence of cross linkers in DNA was eliminated by a study of artificially (mitomycin) cross-

linked HeLa DNA. Although a large fraction of the DNA is made to renature spontaneously by treatment with mitomycin, electron microscopy of samples prepared in 90% formamide show a large proportion of X and Y forms, whereas untreated zero time bound DNA shows only linear molecules as predicted.

Observation by electron microscopy of spontaneously renaturing DNA after treatment with a single strand specific nuclease allows measurement of the lengths of the inverted segments. Such measurements show duplex lengths of 300 to 1,200 nucleotide pairs with a very few as long as 6,000. If zero time binding is caused by inverted sequences forming hairpins, then treatment with single strand nuclease, after prior removal of single strand ends, followed by denaturation would be expected to reduce the average molecular weight to half by digestion of the unpaired terminal loop t and also to reduce zero time binding. That neither of these predictions is fulfilled suggests that the terminal loops are resistant to nuclease probably because they are very short. Electron microscopy confirms this since no terminal loops are visible. The thermal stability of HeLa palindromes ($T_m 81.9^\circ\text{C}$) suggests fidelity of base pairing and consequently low levels of base substitution in the inverted segments. Moreover, base composition analysis (43% G+C) of HeLa hairpins shows that they have the same overall composition as total HeLa DNA.

Wilson and Thomas were able to estimate the distances separating adjacent palindromes by measuring molecules in electron micrographs and found that they are located in clusters of two to four. Individual palindromes are separated by predominantly $0.7\ \mu\text{m}$ although some are $8.5\ \mu\text{m}$ apart, but the clusters of palindromes are more sparsely distributed ($40\ \mu\text{m}$). The average spacing of hairpins can also be estimated from the slope of the curve of amount of spontaneously renaturing DNA against fragment size and shows that in HeLa cells palindromes occur every $13.5\ \mu\text{m}$ on average and they are more sparsely located in mouse L cells ($62\ \mu\text{m}$) and *Drosophila* ($75\ \mu\text{m}$). Finally, Wilson and Thomas produce evidence to suggest that the sequences flanking palindromes in mouse L cells are not a random sample of all mouse DNA sequences but are selected. When ^3H -labelled zero time bound DNA is hybridised in the presence of ^{32}P -labelled non-bindable DNA to filters containing unlabelled zero time bound DNA in conditions in which the palindromes have renatured before the reaction, observation of the renaturation of adjacent sequences is possible. Zero time bound DNA preferentially hybridises to ^3H -labelled zero time bound

DNA and not to the ^{32}P -labelled non-bindable fraction. It seems therefore that palindromes are located non randomly in the genome.

Although the evidence for the existence of palindromes is quite good, there is little idea about their functions. Theoretically a palindrome in native DNA may exist in two forms—the normal linear double helix or a 'cruciform' in which the strands have opened and formed two hairpins. A search for 'cruciform' structures in native DNA revealed nothing, however, and thus if they exist they must occur transiently or be stabilised by chromosomal proteins. One clue to their function may be gleaned from the observations on recognition sites in some nucleic acid-protein interactions; for instance, restriction enzymes and the *lac* repressor recognise relatively short palindromes. Furthermore, hairpin loops exist in transfer RNA, ribosomal RNA and perhaps messenger RNA and heterogeneous nuclear RNA and may be responsible for some of the specificity of interaction of these RNA molecules with the appropriate proteins. It is clear that as usual in science description of a phenomenon has outstripped analysis of its physiological importance.

Model interneurone in a locust

from our *Insect Physiology Correspondent*

ONE of the best known of all interneurons is the 'descending contralateral movement detector' of the locust *Schistocerca gregaria*, which is located in the brain and responds to movement in the visual field of the eye on the opposite side of the head. It is particularly responsive to small dark objects the abrupt movement of which evokes a strong brief excitation and a

more prolonged inhibition, associated with the rapid jump of the locust, mediated by motor neurones in the metathorax. O'Shea, Rowell and Williams (*J. exp. Biol.*, **60**, 1; 1974) now provide an excellent example, of the way in which modern neuroanatomical techniques can be applied to the insect central nervous system in order to obtain visual preparations and intracellular recordings of neurones known previously only from extracellular records.

The position of the axon was determined by splitting the axon bundles and checking the locations of the well known response characteristics of the axon under study by extracellular recordings. Probing with microelectrodes was then continued and when the unit was penetrated and its identity confirmed by its response characteristics, the axon was marked by injection of cobalt ions through the recording electrode. Iontophoresis of cobalt ions into the axons isolated by cord splitting, followed by application of ammonium sulphide, leads to the visualisation of the axons and their branches in whole cleared ganglia.

The same procedure of microelectrode penetration of the various brain neurones until the characteristic response of the descending contralateral axon was evoked, followed by marking of the cell body by electrophoretic injection led, in favourable preparations, to the filling of the entire neurone and thus to confirmation of the results of iontophoresis of the descending axon from its lower end, and to the demonstration of its thoracic connections.

The cell body of this model interneurone is the largest in the brain, $45\text{--}50\ \mu\text{m}$ in diameter. Earlier electrophysiological studies by Burrows and Rowell had shown that it makes

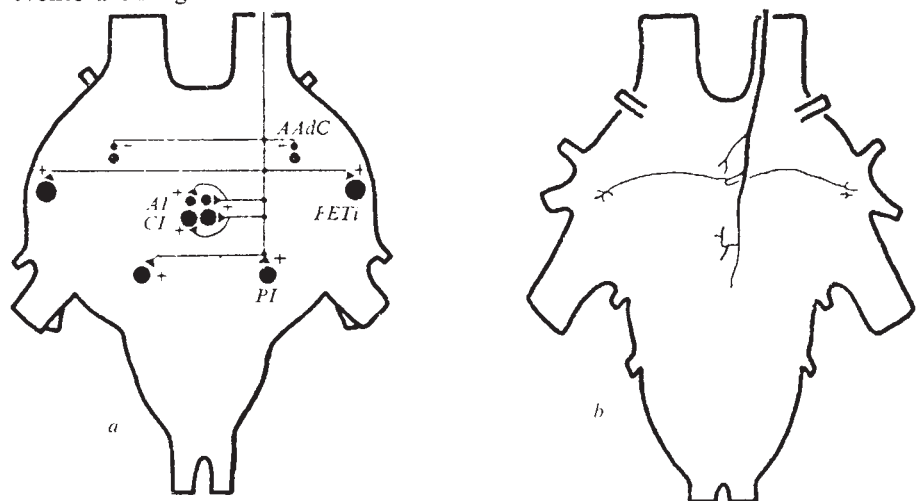


Fig. 1 *a*, Distribution of the descending contralateral movement detector neurone in the methothoracic ganglion of the locust as inferred from electrophysiological recordings from motor neurones by Burrows and Rowell. The motor neurones are labelled: AAdC, anterior coxal adductor; FETi, fast extensor tibiae; AI, anterior inhibitory flexor tibiae; PI, posterior inhibitory flexor tibiae; CI, common inhibitory. *b*, The distribution as visualised by cobalt injection.

bilateral connections with various motor neurones in the metathorax (Fig. 1a) ensuring rapid extension of the tibia, with inhibition of the antagonistic flexor muscles and of other systems. The distribution of the axon in the metathorax as revealed in the paper under review (Fig. 1b) shows striking agreement with the conclusions reached by electrophysiological methods alone. There are no major branches to be seen in the metathoracic ganglia which cannot be accounted for by the known connections with motor-neurones involved in the jump. It seems likely that this is the most important function of the descending movement detector neurones. There are also restricted connections with neurones in the prothorax and mesothorax, whose role in the jump are at present unknown. There are considerable individual differences in the detailed arrangements of these neurones. Assuming, as the authors do, that these differences are genetically determined, they may well form the basis of a behaviour variance, in the population, subject to natural selection.

O'Shea *et al.* conclude by pointing out that by using the methods of iontophoresis of cobalt ions, neural anatomists could uncover rich new fields for physiological investigation.

Mapping of poliovirus receptor

from our
Molecular Genetics Correspondent

POLIOVIRUS provides one of those interesting situations in which the susceptibility of a cell to a virus may depend upon its possession of the appropriate receptor. Human cells possess the receptor and can therefore be killed by the virus; whereas rodent cells usually cannot be infected. But in human-rodent hybrid cells, possession of a human gene for the poliovirus receptor is sufficient to enable the virus nucleic acid to enter the cell and, once this first step has been taken, the virus can then multiply without the mediation of any further human gene products, the rodent genetic apparatus being sufficient for its needs. It should therefore in principle be possible to identify the human chromosome(s) carrying the gene(s) concerned with poliovirus reception by making use of human-rodent hybrids which differ in their complement of human chromosomes; when some of the human chromosomes have been shed after hybridisation, loss of the appropriate human chromosome(s) should mean that the hybrids also lose their susceptibility to poliovirus. By performing such experiments, Miller *et al.* report in the April issue

of *Cell* (1, 167; 1974) that a single human chromosome is responsible for conferring upon cells the ability to accept poliovirus.

These experiments have succeeded where earlier attempts to gain this information failed because of two recent developments; hybrid lines are now available with a high degree of homogeneity of human chromosomes (necessary to establish correlations with poliovirus sensitivity) and banding techniques can be used to identify these chromosomes from the others of the set. Virus-resistant and virus-sensitive human-mouse hybrid cells all proved to possess about the same number of human and mouse chromosomes, excluding the possibility that changes in the ratios of two genomes might be relevant in establishing susceptibility to the virus (a mechanism important in polyoma susceptibility or adenovirus multiplication).

The chromosomes of several poliovirus resistant and sensitive lines were examined by both fluorescent Q banding, which produces characteristic striations of each human and mouse chromosome, and also by C banding, which stains principally the centrometric heterochromatin. Cell lines resistant to poliovirus lacked human chromosome 19, whereas lines sensitive to infection possessed this chromosome. This implies that chromosome 19 carries all the information necessary for acceptance of poliovirus by a human cell. This does not prove, of course, that only a single gene codes for the receptor protein; but this is the most likely interpretation of these results since the genes carried on several chromosomes were responsible, the loss of each of these chromosomes might produce the receptor-deficient phenotype. The idea that more than one gene might be involved, but that all may be located on chromosome 19, may be excluded in future experiments.

Interference in heavy ion inelastic scattering

from our
Nuclear Theory Correspondent

ONE OF THE most interesting features of heavy ion interactions is the interference effects between the different processes that can take place when the interacting ions differ by only a few nucleons. For example, if ^{16}O is scattered by ^{17}O , the neutron transfer reaction from ^{17}O to the ground state of ^{16}O gives the same emerging particles as the simple elastic scattering and so is indistinguishable from it. The corresponding amplitudes interfere quantum mechanically and if they are of comparable

New MRC unit

THE Medical Research Council set up on April 1 a Mammalian Development Unit in University College, London. Its head is Dr Anne McLaren who previously worked in the Agricultural Research Council Unit of Animal Genetics in Edinburgh. She will be assisted initially by three other senior scientists two of whom are Dr M. H. L. Snow, also from the ARC Unit of Animal Genetics, and Dr D. G. Whittingham who is at present attached to the Physiological Laboratory in Cambridge.

The unit will study the growth and differentiation of the mammalian embryo, concentrating on the expression of genes in the early post-implantation embryo. Some of this work will be done in collaboration with Professor H. Harris. Dr McLaren will continue to use mice as her main experimental animals.

Dr Whittingham plans to extend his work on the low temperature storage of mouse embryos in collaboration with Dr Mary Lyon. The practical application of this work is in livestock breeding but many experiments, such as those on the mutagenic effects of irradiation on the stored embryos, can only be done with an animal whose genetics are well understood.

The new unit is being housed in the former home of Professor H. Grüneberg's Experimental Genetics Unit which was disbanded in 1972. Its budget is £175,000 for five years, unless it expands quickly enough to need more.

magnitude this is evident in the observed elastic scattering cross sections.

Interference phenomena are sensitive to the relative amplitudes and phases of the contributing process so they can provide a detailed check of the models used to calculate them, in this case of the distorted wave theory of the interaction and the optical potentials used, as well as of the spectroscopic factors used to obtain the transfer amplitude. Many studies of elastic scattering of similar ions are being made with these aims in mind.

A group at the Max Planck Institute for Nuclear Physics in Heidelberg (Gelbke, Baur, Bock, Braun-Munzinger, Grochulski, Harvey and Stock, *Nucl. Phys.*, **A219**, 253; 1974) has recently obtained evidence for the occurrence of similar interference phenomena in the inelastic scattering of similar heavy ions. They bombarded ^{16}O with ^{17}O ions with energies ranging from 22 to 32 MeV and measured the cross section for the

reaction leaving a ^{17}O ion in its $\frac{1}{2}^+$ state at 0.871 MeV.

This process can take place in at least two ways. First, the energy of the interaction can simply promote the odd neutron in ^{17}O from its ground state orbit to the one corresponding to the 0.871-MeV state; this is the normal inelastic scattering mechanism. But an experimentally indistinguishable result is obtained if the odd neutron in the incident ^{17}O ion is transferred to the orbit in the target ^{16}O that leaves the resulting ^{17}O nucleus in its 0.851-MeV state. This process gives a cross section that is peaked in the backward direction whereas the former process gives one peaked in the forward direction. At the energies of this experiment both amplitudes have broad angular peaks and similar absolute magnitudes, so significant interference effects can occur.

These cross sections were calculated using the distorted wave theory and the results obtained for the dominant transfer inelastic process above are shown in Fig. 1. The overall behaviour of the cross sections is given quite well but close examination shows fine structure in the data that is not reproduced theoretically. Additional calculations were then made including the contribution from the direct inelastic scattering process, and the results (Fig. 2) show that the fine structure is at least qualitatively reproduced. A perfect fit is not to be expected because higher order processes are not taken into account explicitly in this work. Some implicit account was taken of them by multiplying the direct inelastic scattering amplitude by a factor of 1.8i and this gives a much improved fit.

This work provides convincing evidence for the presence of interference

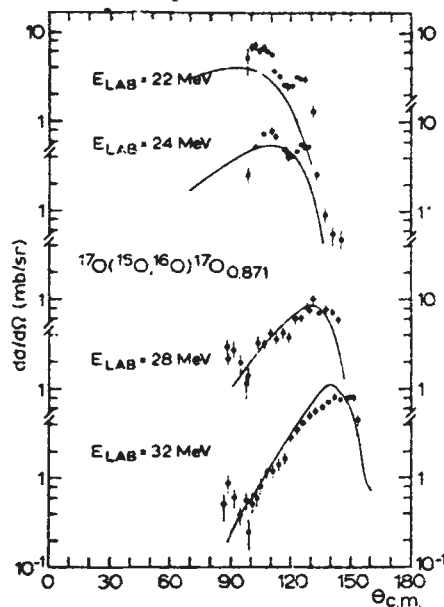


Fig. 1 Differential cross sections for the inelastic scattering of ^{16}O by ^{17}O compared with distorted wave calculations of the one-neutron transfer process.

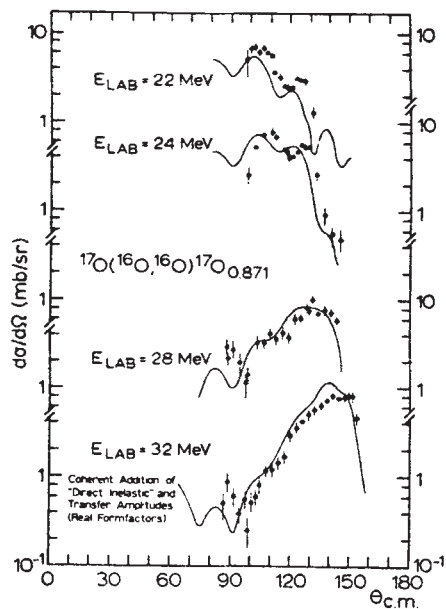


Fig. 2 Differential cross sections for the inelastic scattering of ^{16}O by ^{17}O compared with distorted wave calculations of the coherent addition of the direct inelastic and inelastic transfer process.

effects in the inelastic scattering of heavy ions and is likely to develop into a useful spectroscopic tool.

Gene isolation and HnRNA

from a Correspondent

HnRNA, an abbreviation for the more cumbersome 'heterogeneous nuclear RNA', has been a difficult, yet intriguing, class of RNA molecules to study. Although it was discovered in 1961, clear and formal evidence that HnRNA is a high molecular weight intermediate, or precursor, in the biosynthesis of messenger RNA has been discovered only recently. The turning point came (as J. Darnell of Columbia University, New York, explained at a symposium of the nucleotide group of the British Chemical and Biochemical Societies held in Glasgow on April 1 and 2) when it was found that both HnRNA and mRNA have the common and distinctive chemical feature of an extensive polyadenylic acid region at one end of the molecule.

These studies were made initially on the complex mixtures of both messenger RNA and of HnRNA present in a widely used human tumour cell line of epithelial origin (HeLa cells). The same type of precursor-product relationship has, however, been convincingly shown by both R. Williamson (Beatson Institute, Glasgow) and K. Scherrer (Institute for Cancer Research, Lausanne) for the biosynthesis of the specific globin messenger RNAs in the specialised red blood forming cells. Scherrer emphasised the complexity of the maturation

process of the highest molecular weight HnRNA to the end product globin mRNA even in this well defined system. The biosynthesis of the heavy chain immunoglobulin mRNAs (A. R. Williamson, University of Glasgow) may be simpler as only two well defined sizes of HnRNA occur.

In the maturation of HnRNA at least 90% of the molecule is degraded in the nucleus. Both Darnell and R. H. Burdon (University of Glasgow) described uridylyate-rich regions in this part of HnRNA. Furthermore, extensively repeated helical regions occur (Darnell).

The control of the synthesis of HnRNA is an area where it is easier to suggest mechanisms than to verify whether these mechanisms actually operate. R. S. Gilmour (Beatson Institute, Glasgow), however, is studying the induction of haemoglobin synthesis in chromatin (thus studying genes which are actively capable of being expressed) in a 'Friend' leukaemic cell, has isolated a mutant with more globin mRNA. This may be a 'transcriptional' mutant with an increased rate of synthesis of HnRNA. By contrast, the absence of α -globin synthesis in a still-born child suffering from *Hydrops foetalis* (an extreme form of α thalassaemia) is caused by the absence of the α -globin gene rather than by any fault in synthesis of mRNA (R. Williamson).

Two speakers, D. D. Brown (Carnegie Institute, Baltimore) and M. L. Birnstiel (University of Zurich) described the isolation and some properties of specific animal genes. Brown has made an extensive study of the reiterated genes coding for 5S ribosomal RNA in the South African toad (*Xenopus laevis*) and he presented new evidence of the size and sequence heterogeneity of the 'spacer' regions which alternate with the coding regions in this extensively repeated gene. Furthermore, recent sequence studies on the spacer have shown that it consists in part of many short identical or nearly identical sequences in tandem, the average repeat length being sixteen bases.

The importance of the radioactive sequence methodology was emphasised by F. Sanger (MRC Laboratory of Molecular Biology, Cambridge) when he described his recent work on sequencing DNA by radioactive methods. DNA may be labelled either 'directly' or by 'copying' methods. The first approach was used to derive two 50-long sequences in the bacteriophage ΦX ; using the second approach he described the methods for extending a primer complementary to the single stranded DNA of bacteriophage f1 using DNA polymerase to deduce a sequence of 81 residues. Undoubtedly the methods will be of very general application to many problems in molecular biology.

Photometry of Comet Kohoutek (1973f)

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Extensive photoelectric and infrared photometry of Comet Kohoutek (1973f) shows that its nucleus is 10 to 15 km in diameter and that the comet contains relatively little dust, and provides new information about the nature of cometary dust grains.

WE observed Comet Kohoutek (1973f) photometrically from 0.3 to 30 μm and at heliocentric distances from 0.37 to 1.9 AU. Short of 1.5 μm , the flux consists of emission lines and sunlight scattered by cometary dust; the observations in this spectral region show that the nucleus is no larger than those of a number of other recent comets. Thermal re-emission

from the dust is dominant beyond 2 μm ; our data show that Comet Kohoutek contains relatively little dust. These two results explain why the comet was much less spectacular visually than had been expected. The data reported here provide the first large, homogeneous set of photoelectric and infrared photometry obtained for any comet, and they provide new information on the nature of cometary dust.

By necessity, most of our observations were made at very large zenith angles and are therefore less accurate than normal photometry. Single measurements are probably uncertain by at least 10% and even greater uncertainties apply to observations near 20 μm (because of the large extinction corrections at this wavelength) and to those made with a

TABLE 1 Photoelectric photometry of Comet Kohoutek (1973f)

Time of observation		r(AU)	Diaphragms (arc s)					Magnitudes					R	I
Date			12	31	48	62	112	157	U	B	V			
October	8.5	1.92			x					12.38	11.71			
October	16.5	1.79		x						12.37	11.83			
October	18.5	1.75				x				11.45	10.62			
				x						12.41	11.65			
October	19.5	1.74					x			10.79	10.08			
						x				11.68	10.92			
				x				12.69		12.63	11.85			
October	22.5	1.68					x			10.66	9.88			
				x						12.36	11.57	10.88	10.53	
October	25.5*	1.63					x			10.21	9.48			
				x						11.95	11.17	10.77	10.40	
			x							14.05	13.04			
October	26.5	1.61						x		10.06	9.24			
							x			10.31	9.53			
				x					12.07	12.14	11.53			
			x						14.11	13.97	13.02			
October	28.5	1.57						x		9.92	9.28			
							x		9.91	10.17	9.54			
				x					11.95	12.05	11.29	10.68	10.22	
October	31.5	1.52						x		9.66	8.98			
							x		9.85	10.31	9.28			
						x				10.80	10.05			
					x					11.07	10.34			
				x						11.90	11.14			
November	1.5	1.50						x	9.00	9.70	8.99	8.83	8.29	
						x			10.38	10.78	10.06			
				x					11.59	11.82	11.15	10.63	10.15	
			x							13.57	12.87			
November	5.5	1.42	x							13.62	12.71			
								x	8.97	9.41	8.71	8.60	8.26	
						x			10.18	10.47	9.73			
									11.50	11.64	10.90	10.47	9.98	
November	6.5	1.40		x				x	9.04	9.37	8.71	8.60	8.24	
									11.56	11.65	10.97	10.52	10.16	
			x						13.51	13.37	12.60			
November	11.5	1.30						x	8.60	8.91	8.18			
November	14.5	1.24						x	8.15	8.68	7.92			
						x			9.44	9.80	9.05			
				x					10.70	10.97	10.28	9.90	9.44	
			x						12.83	12.77	12.09			
November	16.5	1.20						x	8.06	8.56	7.87	7.94	7.42	
				x					10.75	11.01	10.22	9.90	9.35	
						x			9.53	9.70	9.00			
November	28.5	0.94						x	7.26	7.67	6.97	7.13	6.43	
						x			8.51	8.76	8.01	7.93	7.27	
				x					9.78	9.98	9.26	9.06	8.48	
December	9.5	0.67						x		6.14	5.76	5.86	5.21	
				x					7.92	8.20	7.60	7.45	6.95	
			x						9.86	10.11	9.43	9.14	8.92	

* Extinction coefficients not well determined.

TABLE 2 Infrared photometry of Comet Kohoutek (1973f)

Time of observation Date		Diaphragms (arc s)			Fluxes (Jy)*									
		13.5	6.8	5.5	2.2 (0.6)	3.6 (0.9)	5.0 (1.0)	8.8 (1.0)	10.3 (1.2)	10.6 (5.0)	11.6 (0.8)	12.6 (1.0)	21 (8)	22.5 (5)
October	7.5			x						1.2	1.2	1.6		
October	8.5			x						1.0	1.0	1.8		
October	16.5			x						1.7			2.8	
October	18.5			x						1.6	1.9		3.6	
October	23.5	x								4.6				
October	24.5	x								4.2				
			x							2.6				
November	1.5	x								5.3				
November	9.5			x			2.3	3.9		5.2	5.5	4.4	7.8	
November	15.5	x								14.8				
				x	0.04		0.43	6.2	10.0	8.0	8.9	8.3	18	25
November	28.5	x					46	56		47	49	36	69	
December	6.5	x				3.6	17	81	120	80	98	85	83	98
			x							52				
December	9.5	x				9.4	27	123	170	165	186	158	140	146
December	13.6	x			4.3	40	100	280	520	360	380	370		
December	18.7			x	11	76	160	320	560	450		340	320	220
December	19.7	x				330				1250				
				x	26	175	360	630	800	670	630	450	550	420

* The measurements are identified by the centre wavelength of the filter in μm , followed by the band width. $1 \text{ Jy} = 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$.

12 arc s beam in the photoelectric region (because of the very diffuse nature of the comet in this spectral range).

Photoelectric photometry was carried out in the *UBVRI* system defined by Johnson^{1,2} (Table 1) and infrared observations were carried out with a number of filters (Tables 2 and 3; the absolute calibration is from Low³). The data are presented graphically in Fig. 1; the infrared data have been normalised to a beam of 13.5 arc s by means of the observed dependence on beam size of the flux at 10 μm . Scans through the nucleus of the comet at 3.6 μm and 10.6 μm (see Fig. 2) show the angular distribution of the flux to be very similar at the two wavelengths and support the use of a single correction to the beam size throughout the infrared.

To interpret these observations, it is important to know the contribution of emission lines to the flux observed in each filter. A spectrum obtained by R. E. White (personal communication) on October 16.5 shows that all the photoelectric photometry near this date should be dominated by the continuum, with the possible exception of the *U* band which may be affected by CN emission near 3,880 Å. The rapid brightening of the emission lines after this date is apparent in the relatively rapid brightening in *U*, *B* and *V*. The *R* and *I* bands should be dominated by the continuum throughout our observations. A spectrum obtained by W. Wisniewski (private communication) on December 10.5 shows that the emission lines near the nucleus are much weaker than the continuum in these two bands. Detailed interpretation of the *UBV* photometry since October must await the availability of more spectroscopic observations; when such data are available, the photometry should make it possible to refer all the measurements to a common region centered on the nucleus.

Swings⁴ has listed a large number of infrared emission lines that might be expected from comets. If these lines were strong, they would be apparent in our photometry; for example, bands of CO, CO⁺, CN, CN⁺ and N₂O should

be included in our measurement at 4.8 μm and few additional lines should be added with the wider bandpass centred at 5.0 μm . The smoothness of the spectrum in this region and between 3 and 4 μm , where many other molecular bands fall, implies that the contamination of the infrared photometry by emission lines is small. Higher spectroscopic resolution near 2.2 μm on Comet Ikeya-Seki (1965f)⁵ and near 2.2 and 3.5 μm on Comet Bennett (1969i)⁶ showed negligible contamination of the broadband photometry at these wavelengths by emission lines. The emission peaks at 10 and 18 μm are also seen in the spectra of many infrared stars and are generally attributed to dust containing silicates. The feature at 10 μm was previously detected in Comet Bennett⁷.

Our photometry allows a revised estimate for the diameter of the nucleus of the comet. The initial value of 50 km was based on photographic measurements and an assumed albedo of 0.25. But reflection from a nucleus of 50 km with an albedo of 0.25 would account for the entire signal observed photoelectrically in October with the beam of 12 arc s. Photographs of the comet at that time show that it had a well developed coma which would account for a substantial part of the flux within this beam; therefore, the diameter of the nucleus must have been overestimated. We have extrapolated our photometry to smaller beams in order to estimate the magnitude of the nucleus toward the end of October. A variety of extrapolation procedures yields a range of $14.5 > m_v > 16$; adopting $m_v \sim 15$ and assuming an albedo of 0.5 (a high albedo has been assumed because of the large ratio of gas to dust in this comet), we arrive at a revised value of 10 to 15 km for the diameter of the nucleus. In other words, the nucleus of Comet Kohoutek (1973) is probably no larger than the nuclei of several recent, bright comets⁸.

For detailed comparison, the infrared spectrum of Comet Bennett (1969i)⁷ is included in Fig. 1. If this spectrum were adjusted downwards in flux by a factor of 30, it would fit smoothly into the progression of spectra for Comet Kohoutek

TABLE 3 Additional infrared photometry on December 19.7

Wavelength	1.6	3.05	3.88	4.8	10.8	17	19	24.5
Bandwidth (μm)	0.3	0.1	0.5	0.6	1.0	2.0	1.0	1.0
Fluxes* (Jy)	10	100	180	290	630	530	550	410

* 5.5 arc s beam

except that the feature at $10\ \mu\text{m}$ and the flux in the region 3 to $5\ \mu\text{m}$ are somewhat stronger in Comet Bennett. Since the dust in the two comets is evidently of roughly similar composition, we can compare the amounts of dust by correcting the fluxes for the differences in field of view and geocentric distance. The result is that Comet Bennett had about 16 times as much dust as Comet Kohoutek.

The original predictions that Comet Kohoutek might have $m_v \sim -8$ at perihelion passage were based on the observations of Comet Bennett, renormalised to allow for a nucleus about two times larger in diameter. Since the amount of matter released by a comet nucleus is proportional to the surface area, the downward revisions in the diameter of the nucleus and in the amount of dust would imply that the visual magnitude of Comet Kohoutek was overestimated by about four magnitudes. This result agrees well with the observed magnitude of Comet Kohoutek and indicates that the corrections we have applied are an adequate explanation for its disappointing performance.

The heliocentric distance of the comet in October considerably exceeds those at which infrared observations of comets were available previously⁵⁻⁹. Our measurements indicate no emission peak at $10\ \mu\text{m}$ at large distances from the Sun. This transition should be confirmed as the comet leaves the Sun; it correlates well with recent calculations that high albedo clathrate grains ejected from a comet nucleus would have long lifetimes until the comet approached to within 1.5 to 2.0 AU of the Sun¹⁰.

O'Dell¹¹ has derived an expression for the albedo of cometary dust grains in terms of the integrated infrared surface brightness and the surface brightness of scattered light in the continuum. Our data are the first which permit an accurate evaluation of this expression; they yield

$$\gamma/(1 - \gamma) = 0.16$$

where γ is the albedo. The mean absorption efficiency of the grains in the visible region is then

$$\epsilon_A = 1.0/[1.0 + 0.16 \alpha(\theta)]$$

where $\alpha(\theta)$ is the ratio of the scattering efficiency of the grains averaged over all angles to the scattering efficiency at the angle of observation, θ . Over the range of θ for our observations, $\alpha(\theta) \gtrsim 1$, so that $\epsilon_A \simeq 0.8$.

The infrared observations also provide information about $\epsilon(\lambda)$, the infrared emissivity of the dust grains as a function of wavelength. The temperature of the grains is determined by $\epsilon(\lambda_{\text{max}})/\epsilon_A$ at the wavelengths, λ_{max} , where significant infrared emission occurs. At a given temperature, the spectrum of the grains is controlled by $\epsilon(\lambda)$. Infrared observations at a single heliocentric distance can be fitted by a variety of emissivity laws if appropriate values are assigned to $\epsilon(\lambda_{\text{max}})$. But observations over a large range of heliocentric distance provide much more information about $\epsilon(\lambda)$, since the evolution with changing temperature of any assumed composition and size distribution of grains must be consistent with the infrared spectra.

Major changes in the nature of the dust grains should be reflected in changes in ϵ_A . Starting in November, when there is sufficient spectral coverage in the infrared to evaluate this parameter, it remains approximately the same through the final photoelectric observation on December 9.5. Observations by Ney and Hefele¹² and Ney and Ney¹³, combined with our data, indicate no major changes in ϵ_A at least to 0.23 AU.

If the nature of the dust grains does not vary, observations over a range of heliocentric distance in principle determine a unique $\epsilon(\lambda)$. As the comet moves from 1.3 AU to 0.2 AU, the equilibrium temperature of the dust grains (defined as the temperature they would assume if $\epsilon(\lambda_{\text{max}}) = \epsilon_A$) varies from 250 K to 600 K. Therefore, the grain temperature through this range of heliocentric distance is determined by the emissivity between 4 and $15\ \mu\text{m}$. We have carried out calculations assuming $\epsilon(\lambda) = a \epsilon_A \lambda^b$ over this spectral interval and requiring that the calculated spectra agree with

the observations at 5 and $12.6\ \mu\text{m}$ (two wavelengths chosen to avoid complications from the weak silicate feature at $10\ \mu\text{m}$). We find that $b \leq 0$ gives a poor fit, particularly to the data at 0.23 AU (ref. 13) and also requires $\epsilon(\lambda) > 1$ near $3\ \mu\text{m}$. Much better results are obtained with $\frac{1}{2} \lesssim b \lesssim 1$; $\epsilon(\lambda)$ then has a value of ~ 0.2 at $10\ \mu\text{m}$. In other words, if the nature of the grains has not varied with changing heliocentric distance between 1.3 and 0.2 AU, their emissivity falls from ~ 0.8 in the visible to a minimum of ~ 0.1 near $5\ \mu\text{m}$ and slowly rises toward longer wavelengths through $20\ \mu\text{m}$. Although we have implicitly assumed that all the dust grains have similar composition, most of these conclusions hold for the average properties of dust made up of several kinds of grains, as long as most of the emission

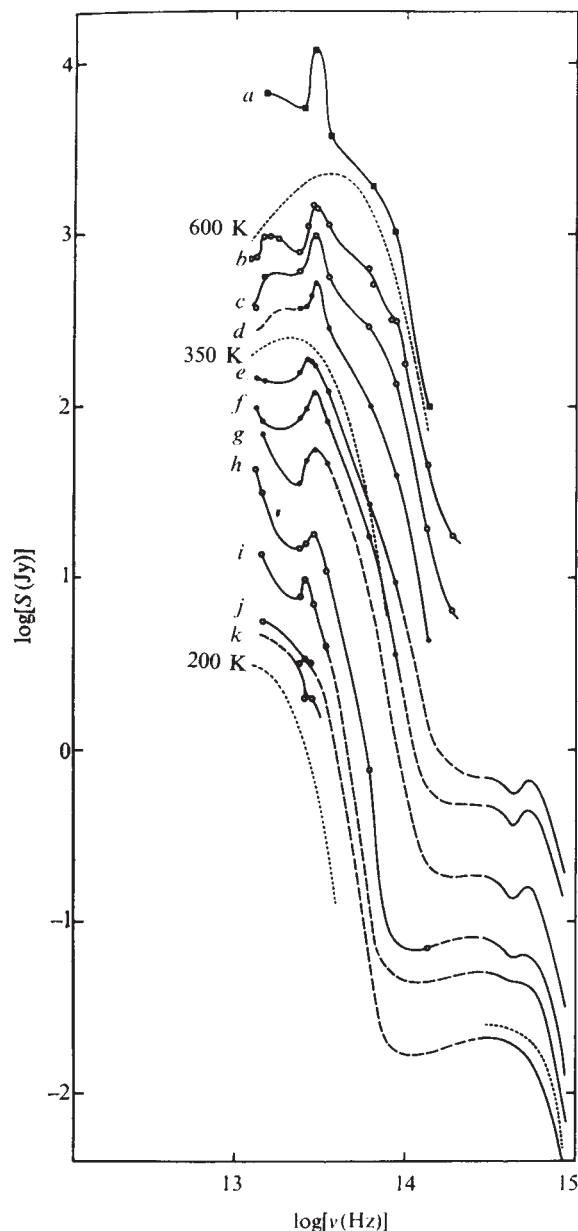


Fig. 1 Photometry of Comet Kohoutek (1973f). The spectra are identified by dates (UT) and heliocentric distances. The spectrum of Comet Bennett (1969i) from Maas *et al.*⁷ is included for comparison. Also shown are a number of blackbody curves in the infrared and a renormalised solar spectrum in the photoelectric region. a, Comet Bennett (0.64 AU); b, December 19.7 (0.37 AU); c, December 18.7 (0.40 AU); d, December 13.6 (0.56 AU); e, December 9.5 (0.67 AU); f, December 6.5 (0.74 AU); g, November 28.5 (0.94 AU); h, November 15.5 (1.22 AU); i, November 9.5 (1.34 AU); j, October 17.5 (1.77 AU); k, October 8.0 (1.95 AU).

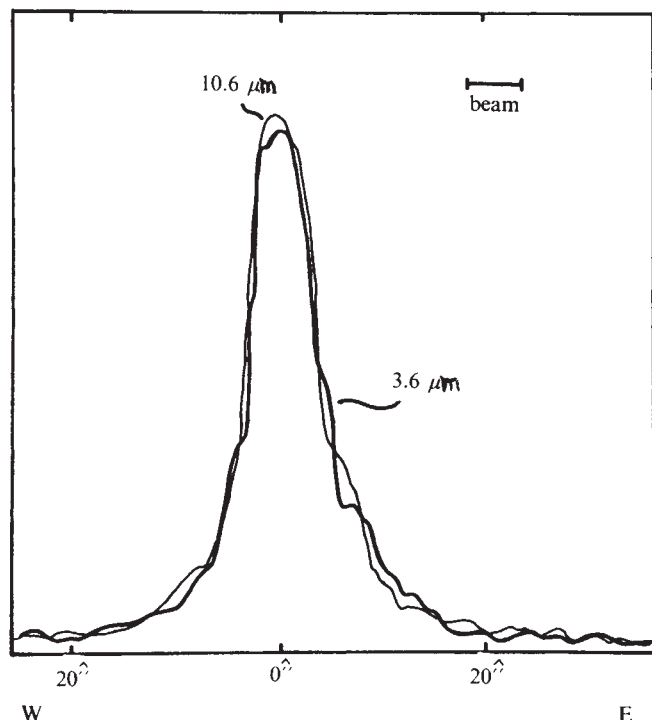


Fig. 2 Scans through the nucleus of Comet Kohoutek (1973f) at 3.6 and 10.6 μm . The observations were made on December 19.7 with a beam of 5.5 arcs.

between 4 and 15 μm is produced by a single type of grain. The scans shown in Fig. 2 imply that a single kind of grain is responsible for the emission at both 3.6 and 10.6 μm , since different spatial distribution would be expected for grains with significantly different properties.

These specific conclusions about the dust grains should

be compared with calculations of the properties of small particles of different compositions, such as those carried out by Krishna Swamy and Donn¹⁴. At present, we do not know of any grain compositions that would have the required properties. Although grains with suitable characteristics may be found, our data favour models in which the emission between 4 and 15 μm is produced by more than one kind of grain or in which the infrared properties of the grains vary between heliocentric distances of 0.2 and 1.3 AU. Models of the first kind must explain the similar spatial distributions of the flux at 3.6 and 10.6 μm , and models of the second kind must be made consistent with the lack of variation observed in the absorption efficiency of the grains in the visible.

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Properties of afferent nerve impulses originating from a neuroma

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Damaged nerves attempt to regenerate. The nerve membrane changes its properties, becomes spontaneously active and may be the source of pain. These impulse generators have unusual properties. They become silent after high frequency activity. This silence may partially explain the effect of counterstimulation as a pain therapy.

WHEN a peripheral nerve is cut, fine unmyelinated sprouts grow out from the central cut ends of the axons. If these sprouts fail to enter the distal part of the cut nerve, they form a tangled mass in the region of the cut. This mass, the neuroma, contains poorly vascularised connective tissue infiltrated with large numbers of the sprouts of the parent axons. The sprouts extend 1-2 mm from the parent axon and they may curve back and enter the connective tissue around the nerve of origin¹. When a neuroma occurs in man

after a peripheral nerve has been cut across and regeneration has failed, it may be a source of pain. Neuromas in man must be the source of nerve impulses and contain mechanoreceptive endings since they are always tender when gently palpated. In some cases, they produce an ongoing barrage of nerve impulses since infiltration of the neuroma with local anaesthetic abolishes pain which these patients refer to the area supplied by the damaged nerve. In spite of the clinical importance and the basic interest of neuromas, physiological studies of them have not been carried out. The physiology of the growing tips of regenerating sensory nerves has been examined² and, unlike normal axons, they were shown to be easily excited by mechanical distortion and by acetylcholine. No mention was made of ongoing activity being generated in the growing tips but it should be remembered that these regenerating fibres are surrounded by their normal surround cells unlike those in a neuroma. Immediately after normal axons are cut across or crushed, a high frequency

injury discharge is generated in all types of nerve fibres but this discharge disappears within seconds and the fibre becomes silent at least for some hours³. The study reported here was designed to investigate what happened some days and weeks after nerve section when a neuroma was established.

An *in vivo*-*in vitro* neuroma

Chronic experiments were carried out on 27 adult rats of the Hebrew University strain weighing 250–450 g. Under anaesthesia, the sciatic nerve was exposed and sectioned in the upper leg. A suture was placed around the cut end of the nerve and 5–8 mm of the nerve was drawn into a close fitting medical polyethylene catheter tube. The distal end of the tube was sealed by heat forming a chamber containing the cut end of the nerve and a length of normal vascularised nerve. The nerve chamber was replaced in the leg and the animal was allowed to recover. On examination of the animal the distal musculature was found to be paralysed and the lateral two-thirds of the foot seemed completely anaesthetised. In 6 of the 27 animals, the animal mutilated the anaesthetised part of its foot after recovery periods of 9–14 d. This phenomenon has been reported before⁴ and may suggest that the animals were suffering some form of anaesthesia dolorosa or phantom pain. If this self mutilation occurred, the animals were at once reanaesthetised, examined and killed but the results did not differ from those from non-mutilating animals who were examined 9–40 d after nerve section.

For the terminal study each animal was anaesthetised with urethane, 750 mg/kg⁻¹, paralysed with gallamine and placed under artificial respiration. The nerve chamber was exposed and microscopic inspection through the wall of the tube showed a terminal neuroma occupying the distal third of the tube with the normal nerve surrounded by loose connective tissue running proximally through the tube and out of the open end. Two 28 gauge hypodermic needles were installed in the distal sealed end of the tube for use as stimulating electrodes and as a way to perfuse solutions through the chamber. The opposite intact sciatic nerve was exposed at the same distance from the spinal cord as the neuroma so that control stimulation could be applied to the intact nerve or to acutely sectioned nerve. For recording, the caudal equina was exposed in an extensive lumbar laminectomy so that the dorsal roots of both sides were available for dissection in an oil pool. Dorsal roots were cut close to the spinal cord and small filaments were dissected free and placed on recording electrodes.

Electrical stimulation

For each animal, a compound potential was recorded on a dorsal rootlet following stimulation of the neuroma (Fig. 1a). For comparison, a stimulus was also delivered at a different time to the dorsal root ganglion region of the dorsal root from which recordings were taken. The same was done on the normal side by recording from a cut dorsal rootlet following stimulation of its ganglion and its intact sciatic nerve at the same distance as the neuroma. The earliest waves on the abnormal and normal sides which result from dorsal root ganglion stimulation were identical and represent the arrival of a volley of which the fastest impulses were conducted at 59 m s⁻¹. When the sciatic nerve was stimulated on the normal side, a delayed compound action potential was recorded because of the longer conduction distance. The earliest component of the action potential was produced by impulses whose overall fastest velocity was 54 m s⁻¹. When the neuroma was stimulated, however, a very delayed compound action potential was recorded even though the conduction distance was identical on the two sides. This compound action potential has two waves, the earliest generated by impulses with an overall fastest conduction velocity of 29 m s⁻¹ and the second with velocities up to 15 m s⁻¹. At

a higher stimulus strength, even slower components were recorded. The delay in arrival of impulses is caused by very slow conduction of impulses in the neuroma (Fig. 2a). Here two compound action potentials are shown; the later one is generated by stimulation within the neuroma and the earlier by stimulating the sciatic nerve 5 mm proximal to the tip of the neuroma. The recording was, as before, on a dorsal rootlet. The fastest impulses from the proximal stimulation arrived at 52 m s⁻¹ but the impulses generated

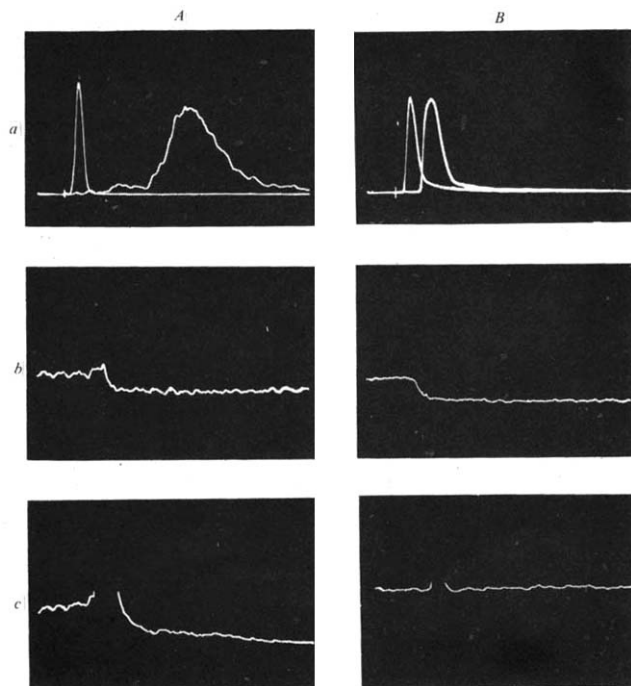


FIG. 1 Recordings from a dorsal rootlet originating from a neuroma (A), and from normal intact sciatic nerve (B). a—Two superimposed sweeps. The early fast wave on the neuroma side and on the normal side were recorded on centrally cut dorsal rootlets following stimulation in the region of the dorsal root ganglion of the recording rootlet, conduction distance 13 mm, fastest velocity 59 m s⁻¹. The slower waves were recorded following stimulation of the normal sciatic at a distance of 45 mm from the recording point and following stimulation of the neuroma also 45 mm from its recording point. The overall fastest velocity from the normal sciatic was 54 m s⁻¹. The velocities for the first components of the two waves originating from the neuroma were 29 and 15 m s⁻¹. Time mark 2 ms.

b—The rate of ongoing activity in a rootlet originating mainly from a neuroma on the left and of ongoing activity in a rootlet originating mainly from an intact sciatic nerve on the right. Some 10 units were being recorded simultaneously with a combined rate of 150 impulses s⁻¹. The output of a rate meter with a time constant of 3.3 s is recorded. At the interruption of the line, 2% lignocaine a local anaesthetic, was perfused through the nerve neuroma chamber in the left record and directly injected into the intact sciatic nerve in the right hand record. Following the injection into the neuroma chamber there is a brief rise of firing rate which is due to the pressure distortion produced by the injection and which was also observed if saline was injected. This is not observed on the normal side. After some seconds on both sides the firing rate drops to 20–30 impulses s⁻¹. The impulses which were not anaesthetised by the local block originated from proximal muscle and not from the normal or abnormal sciatic nerve. Time mark 24 s.

c—Recording as in (b) of the rate of ongoing activity in rootlets originating from a neuroma and from normal sciatic nerve. At the interruption of the line, the central cut end of the rootlet was stimulated at 100 s⁻¹ with maximal 0.1 ms square waves for 6 s. During this period antidromic impulses swept past the recording electrodes and entered the peripheral nerves. After the end of the tetanus, the firing rate of impulses from the normal endings rapidly returned to their control level. But, the impulses from the neuroma were depressed for a long period after the end of the tetanus.

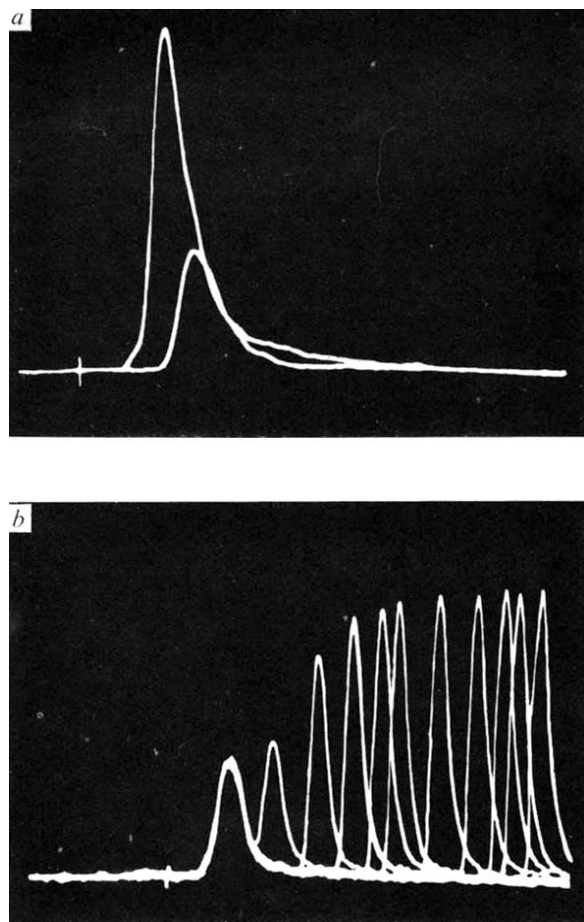


FIG. 2 *a*, Recording from a dorsal rootlet whose axons end in a sciatic nerve neuroma. Two superimposed sweeps. The slower small wave followed stimulation of the distal tip of the neuroma. The faster wave was produced by stimulating the sciatic nerve 5 mm proximal to the distal tip of the neuroma. The time difference between these two waves implies a fastest overall conduction velocity in the terminal 5 mm of 7.5 m s^{-1} . Time mark 2 ms. *b*, Interaction between the fast and slow waves shown in (*a*). Ten superimposed sweeps. For each sweep, a stimulus was given first to the extreme distal part of the neuroma and at varying time intervals following this a stimulus was given to the nerve 5 mm proximal to the neuroma tip. It will be seen that when the second wave occurs less than 3 ms after the first, it is reduced in height because the axons are partially refractory due to impulses in the same fibres which carried the earlier wave.

5 mm in a more distal direction were delayed by a further 0.66 ms giving an overall fastest conduction velocity for this terminal part of nerve and neuroma of 7.5 m s^{-1} . Since the individual fibres in the neuroma turn and twist, it is not possible to state the exact conduction distance nor the location of the stimulation. Furthermore part of the 5 mm length in the chamber is made up of relatively normal fibres. We cannot therefore give the exact conduction velocity of fibres within the neuroma but it is evident that the fibres are electrically excitable and must have a low conduction velocity, considerably below 7.5 m s^{-1} .

Evidence that the small slowly conducted wave originating from the neuroma is carried by the same fibres as the large rapid wave originating from intact proximal nerve is shown in Fig. 2*b*. Here the stimulus to the neuroma is followed at various time intervals by a stimulus to the nerve 5 mm proximal to the end of the neuroma. The recording is made from a dorsal rootlet. Ten superimposed sweeps are shown with the interval between the two stimuli changed for each sweep. The large fast wave is reduced when it approaches to within 2–3 ms of the smaller slow wave. The reason for

this reduction in height is because the impulses from the neuroma cause the axons of the central part of the nerve to be refractory. Therefore the two waves are carried in the same fibres.

Ongoing activity

If fine filaments of dorsal rootlet were teased free and mounted on recording electrodes, it was possible to record small numbers of units and to separate single units by spike height with a window discriminator. The rest of this communication deals with the responses of single axons or small groups of axons originating in neuromas. Filaments were selected which responded after electrical stimulation of the neuroma. These filaments contained fibres which were continuously active in the absence of an intentional stimulus. These impulses originated from the neuroma since they disappeared when the nerve-neuroma chamber was perfused with 2% lignocaine to produce local anaesthesia in the chamber (Fig. 1*b*). These continuously active fibres were probably myelinated afferents of relatively small diameter because the spike height of the units was less than one-third the height of the large spikes originating from muscle spindles and it is known that there is a correlation between spike height and fibre diameter⁵. If slight pressure was applied to the neuroma either by distortion of the chamber or by saline injection, the continuously active units increased their discharge rate. Previously silent fibres with higher spike heights showed a brief phasic burst of spikes during the onset of the pressure increase. Unmyelinated afferents were not examined. Filaments of ventral roots whose axons terminated in the neuroma were examined and were found not to contain ongoing antidromic impulses from the neuroma. Antidromic impulses could however be produced in these ventral root fibres by pressure on the neuroma.

It was noted that if the neuroma was driven to a high level of activity by direct repetitive electrical stimulation, the ongoing activity ceased after the stimulation. This is a phenomenon of considerable interest but since direct stimulation might have complex local effects under the stimulating electrodes, a method of activating the terminals was used in which only antidromic impulses were used. The central cut end of the filament from which recordings were made was placed on stimulating electrodes while recording electrodes were placed more distally on the same dorsal root filament.

The impulses originating from the neuroma were recorded and fed into a rate meter and a control period was recorded (see Fig. 1*c*). The filament was then stimulated maximally with 100 s^{-1} 0.1 ms square waves for 6 s. This tetanic barrage of nerve impulses swept antidromically past the recording electrodes and entered the neuroma. Following this tetanus, it will be seen that the rate of discharge from the neuroma fell and remained low for the duration of the record. The rate of spontaneous firing was depressed for minutes in all units examined and for some units, the resting discharge did not return for more than an hour. These prolonged suppressions of firing were never observed from any intact sensory endings whose excitability and ongoing discharge returned to control levels within seconds of such an antidromic invasion.

Since a tetanus is known to hyperpolarise central terminals^{6,7}, it was thought possible that this also occurs with a high-frequency antidromic invasion of a neuroma. This was tested by electrically stimulating the neuroma at 2 s intervals with a submaximal stimulus and recording the height of the orthodromic compound action potential. It was found that the electrical excitability of the neuroma decreased following a 10 s antidromic invasion at 100 s^{-1} . This decreased excitability lasted between 50 and 110 s whereas the decrease of spontaneous activity lasted in these cases between 2 and 6 min. The shorter duration of the assumed hyperpolarisation of the neuroma compared with the dura-

tion of the decreased spontaneous activity does not necessarily mean that the two are unrelated because the electrical stimulation may have triggered impulses in more proximal and larger axon sprouts than the finer more distal regions from which the impulses might originate. It might be expected since the nerve sprouts in the neuroma are fine and intertwined that they might make contact with each other so that ephaptic interactions might occur between the fibres. To test this, recordings were made from one dorsal root filament while maximal single or tetanic stimuli were applied to the entire remaining dorsal root so that the neuroma would be invaded by a large mass of nerve impulses in many fibres other than the nerve fibres from which recordings were being made. No signs were observed of any excitation or change of the spontaneous activity in fibres which had not carried the antidromic volleys and it is apparent that no obvious interaction occurs between the sensory fibres in a neuroma. Similarly, maximal stimulation of a ventral root failed to produce an afferent discharge in the dorsal root or *vice versa*. A final difference of the generation of nerve impulses from a neuroma in contrast to normal spontaneously active endings was that the discharge stopped within seconds of cardiac arrest whereas normal endings continue to discharge for many minutes.

Therapeutic implications

It is evident that these neuromas were the source of a steady barrage of nerve impulses in certain nerve fibres and that the generator of these impulses showed unusually fragile properties, being inactivated by a tetanus and being highly dependent on blood flow. The preparation with its possibilities for perfusion is an *in vitro-in vivo* isolated group of nerve endings in which it should be possible to study the action of the ionic and chemical environment on impulse generation. In addition, the results have certain therapeutic implications. If nerve impulses of the type reported here are a source of pain in man with damaged peripheral nerves and if the generator of these impulses has a lower safety factor than that of impulses from healthy nerve endings, then it is possible that drugs might be developed which affected the pathological generator without affecting normal tissue. Of more immediate interest is the use of electrical stimulation of peripheral nerve for the control of pain. This was initiated in 1967⁸ and is being widely developed. The rationale for the therapy was the suggestion that massed stimulation of peripheral fibres of large diameter set up central inhibition and closed a central gate mechanism⁸ which decreased the

transmission from the periphery to central pathways of impulses which generate pain. It was shown that certain patients were relieved of their pain not only during the tetanic submaximal stimulation of their peripheral nerves but for long periods after the end of the stimulation whereas other patients were relieved only during stimulation. The long lasting relief was a puzzle because no prolonged inhibition of spinal cord transmission was observed in animals after stimulation ceased. Now we may have an explanation. It may be that the prolonged relief which occurred in patients with damage to peripheral nerves was not produced by central inhibition but by the antidromic invasion of the damaged nerve which contained fragile generators of impulses of the type reported here. The suggestion has been made before that part of the analgesia produced by peripheral nerve stimulation was due to peripheral nerve block⁹. This suggestion was, however, based on studies of normal subjects in which, in spite of the extremely intense (20 times threshold) stimuli which were used, no clear evidence was presented that the periphery was in fact blocked. This raises the question of how such intense stimuli are tolerated and the most likely explanation is that the preceding and concomitant stimulation of large fibres sets up central inhibitions which reduces the transmission of impulses from smaller pain-producing fibres. There is considerable physiological evidence that central inhibitions are generated by repetitive peripheral nerve stimulation¹⁰. It is now apparent, however, that in certain patients who respond with prolonged relief to stimulation of a damaged nerve and who show no relief when neighbouring nerves are stimulated, there may be a turn-off of a peripheral source of pain as well as some central inhibition.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Seismic source in East Kazakhstan

ON May 1, 1969 a seismic event occurred in East Kazakhstan which, on the widely accepted body wave magnitude (m_b): surface wave magnitude (M_s) criterion^{1,2}, would be identified as an explosion; $m_b = 4.9$ and $M_s = 2.4$ (ref. 3), giving a difference of 2.5, which is typical of explosive sources. For earthquakes $m_b - M_s$ is usually less than 1.0 magnitude unit. At several teleseismic stations the P seismograms from this event recorded two clear arrivals separated by 7.5 s, which

might indicate two explosions fired in succession. At some of these stations, however, the second arrival is clearly of opposite polarity to P and is thus probably the surface reflection pP, indicating a depth of focus of about 25 km. As this depth is outside the range of routine drilling techniques the event has been tentatively identified as an earthquake³. We show here that it definitely was an earthquake.

Figure 1 shows the P seismograms (*b*, *c*, *h*) for the event, from Yellowknife, Canada (YKA), Warramunga, Australia (WRA) and Gauribidanur, India (GBA). The YKA and WRA seismograms show two arrivals 7.5 s apart; at WRA the second arrival seems to have reversed polarity relative to P, but the YKA record shows no obvious reversal. Deconvolving to remove the effects of anelastic attenuation and

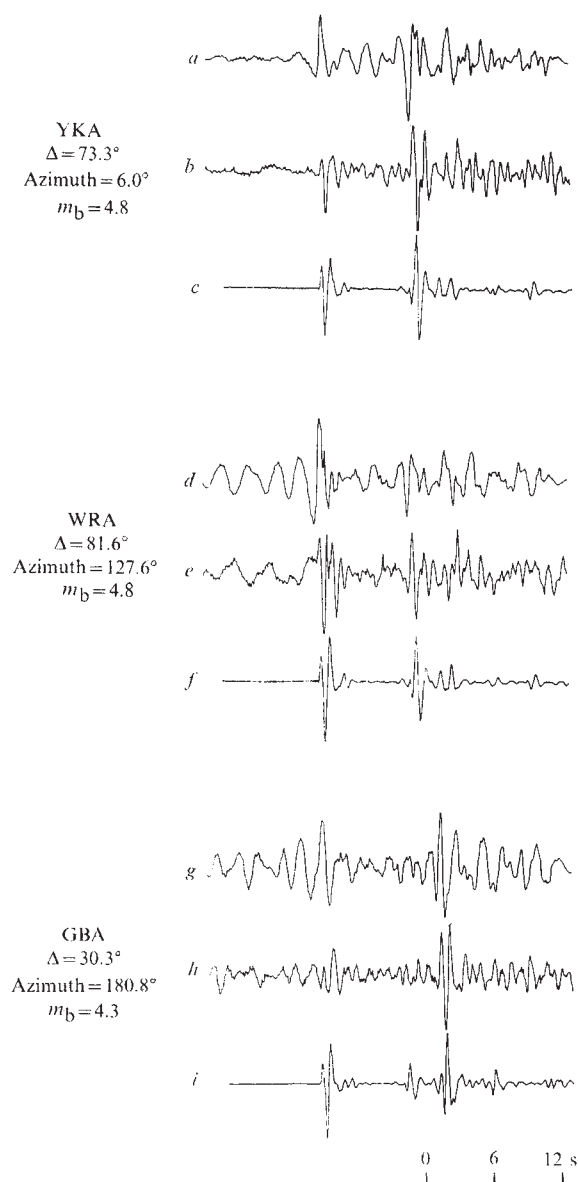


FIG. 1 Seismograms for the East Kazakhstan event of May 1, 1969 (origin time 04 h 00 min 08.7 s). YKA, Yellowknife: *a*, deconvolved trace; *b*, observed trace; *c*, theoretical trace. WRA, Warramunga: *d*, deconvolved trace; *e*, observed trace; *f*, theoretical trace. GBA, Gauribidanur: *g*, deconvolved trace; *h*, observed trace; *i*, theoretical trace. Theoretical seismograms were computed for a dip-slip double couple source, with fault plane dipping at 50° towards YKA.

of the recording system⁴ shows, however, that the second arrival is clearly reversed relative to the first at both YKA and WRA (Fig. 1*a* and *d*) and is thus probably pP. The deconvolution was made assuming a value of $T/Q = 0.2$ s, where T is the travel time and Q the attenuation factor.

The seismogram from GBA, although rather noisy, also shows two arrivals, but the separation of these is about 10.0 s, so that the second arrival at GBA cannot be pP if that arrival has been correctly identified at YKA and WRA. At GBA pP must be absent or at least very small. The deconvolved GBA record is rather difficult to interpret because of the noise but again it seems that the second arrival is reversed relative to the first.

As the second arrivals at all three stations are of reversed polarity relative to the direct P, they are almost certainly surface reflections. If so, the depth of focus is too great for the source to be an explosion. We can however obtain further confirmation that the event is an earthquake, by modelling the seismograms. We have described⁵ the use of this technique

to model the YKA seismogram for another explosion-like earthquake which occurred about 100 km from the event of May 1. We showed that this YKA seismogram could be understood by assuming that the earthquake source was a dip-slip fault of finite size with fault plane dipping at about 50° to YKA. On this model the computed seismogram agrees closely with the observed seismogram. The YKA seismogram for the May 1 event is similar to that used in our earlier study, suggesting that the source mechanisms of the two events are similar. We have in fact used exactly the same model to compute theoretical seismograms for the May 1 event as we used earlier, except that in order to fit the P-pP delay time, the source depth has been increased from 19.4 km to 25.4 km. In addition the depth of the Moho has been increased from 23.4 km to 31.6 km, to keep the source in the crust. Figure 1*c*, *f* and *i* shows the theoretical seismograms. Agreement between theory and observation is good; in particular the model predicts the large pP at YKA and WRA and the small pP at GBA. The large arrival at GBA 10 s after P is evidently sP as predicted by the model. Because PeP is not included in the theoretical calculations, its presence in the observed seismograms should be ignored when comparing theory and observation.

Finally, the deconvolved seismograms show that the shapes of the P and pP pulses at YKA are different (Fig. 1*a*): for P the leading edge is steeper than the trailing edge, whereas for the pP the reverse is true. These differences in shape between P and pP can be explained in terms of a spreading fracture (see ref. 5). For an explosion, P and pP should have the same shape. On the simple source model assumed here, differences in pulse shape similar to those seen at YKA should be seen in reverse at GBA, which is on an opposite azimuth from the source. Furthermore the trailing edge of P should be sharper than the leading edge and *vice versa* for sP. In fact the deconvolved seismogram for GBA does not show this predicted effect. In the source model used, however, the fracture spreads symmetrically from a point of initiation in the centre of the fault plane and this is probably a simplification which needs further study.

Nonetheless, from the evidence of the relative amplitudes and the polarity of P, pP and sP at YKA, WRA and GBA, and the pulse shapes of P and pP at YKA, we conclude that the event of May 1, 1969 is an earthquake and not two explosions. This leaves the large difference $m_b - M_s$ of 2.5 unexplained; models of the type described here have $m_b - M_s$ differences of around 1.2 (ref. 5). A detailed study of the surface waves might suggest a reason for the discrepancy but such a study is virtually impossible in this case because the wave trains are mixed with the surface waves from a large ($M_s = 5.6$) Tongan earthquake. Because of this mixing it is not even certain that the M_s estimates for the earthquake of May 1 are reliable.

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Cyprus: seismic studies at sea

THE experiments described here were carried out in October 1971 on MV *Researcher* during a cruise in the eastern Mediterranean with personnel from the Department of Geodesy and Geophysics, University of Cambridge. Figure 1 shows the location of reflection surveys G and H, planned as a detailed study of the south-western boundary of the alleged Turkish plate, which is drawn by McKenzie as an arc around the south side of Turkey^{1,2}. Two short refraction lines X and Y were placed in Famagusta Bay and Morphou Bay respectively to trace the extent of the rock formations already distinguished by seismic methods on land⁴.

The reflection system comprised a 30 cubic inch air gun fired at 2,200 pounds per square inch at 10 s intervals into a Géomécanique hydrophone array with three active sections each 100 m long. An 8 kJ sparker was also used. The refraction experiments used the single ship radio sonobuoy technique with Cambridge radio telemetric buoys⁵.

The surveys carried out close to Cyprus penetrated only 0.6 s of sediment during sparker runs on G₁, G₂ and Grid H, but reached 2.5 s when the air gun was used on G₁ and G₂ (Fig. 1) (two-way times are quoted throughout). On Grid G there is an undulating upper reflector, A, which occurs within the unconsolidated sediments. It lies above a horizon, B, which is disturbed by folding, and which is 1.5 s below the sea floor. Below this there is a more intensely folded horizon, C, at 2.5 s (Fig. 2). B is probably the top of the 3.0 km s⁻¹ layer observed on refraction line X in Morphou Bay, and C can be correlated with the top of a 4.4 km s⁻¹ layer previously recognised as a limestone bed⁶, but correlated here with pillow lavas (Table 1).

Sediment thicknesses seem to be greater in the north and east towards the continental rise of the Gulf of Antalya, where deep basins occur. There is evidence of quite recent tectonism in this area, with depositional and compaction processes tending to smooth out the surface topography. There are some rather unusual topographic features along G₄: lenses or domes 2–6 km long show internal stratification, and the adjacent sediments are transparent (Fig. 3). It is possible that these domes represent olistoliths transported over a long distance from the Turkish shelf. Alternatively, they may be local sediment lenses, isolated by current action.

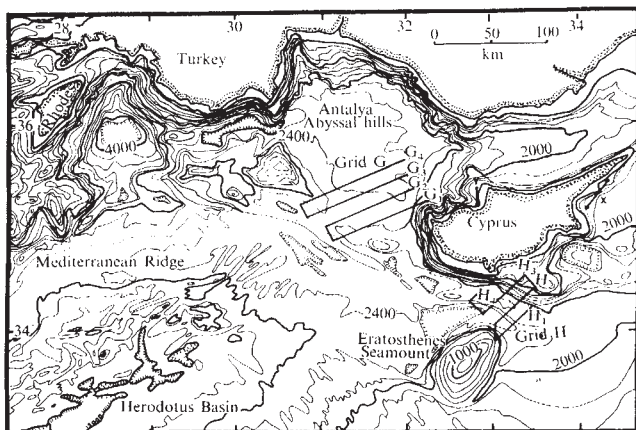


Fig. 1 Location of seismic experiments near Cyprus, superimposed on bathymetry of Carter *et al.*, 1972 (ref. 3). Contour interval 200 m.

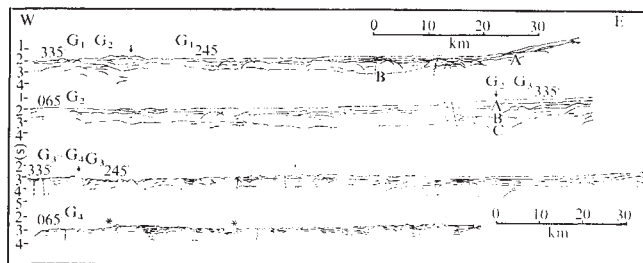


Fig. 2 Line diagrams of reflection profiles on survey area G drawn from west to east. Two-way times are in s, the arrows mark course alterations.* Enlarged section shown in Fig. 3.

Further to the east, transparent knolls 10–40 m high alternate with stratified subsiding basins. The layers thin out against the steep margins of the knolls, indicating that the latter are rising, so it is possible that their origin is diapiric. In this case, the other stratified domes may be areas of marginal sediment deposition which have been isolated by the subsidence of larger knolls or possibly saliferous diapirs 8 km across, due to solution effects, and thus producing the features shown in Fig. 3. Over Grid H a sequence of basins and swells similar to that of Grid G can be identified (Fig. 4) where the rocky basement is first submerged below ponded sediment and then rises to form a hummocky bot-

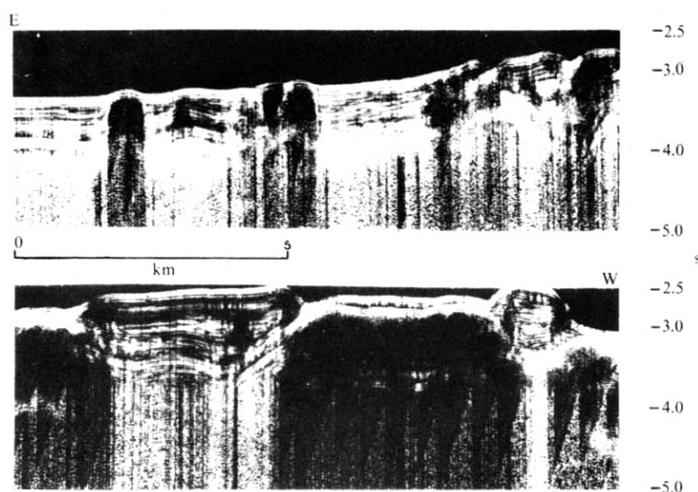


Fig. 3 Topographic features on G₄ showing diapiric type structures, and stratified domes to the west. The position of the section is marked* in Fig. 2. Two-way times are shown.

tom topography. Two reflectors are recognised at the north-eastern end of lines H₁, H₂ and H₃, which may correspond to the upper boundaries of the layers with refraction velocities of about 3 and 4 km s⁻¹ observed on refraction lines X and Y.

A correlation of the areas of deformation occurring over survey areas G and H was attempted, and it seems that a zone of deformation exists on the western side of the lines, which coincides with the areas of deepest water (Figs 1, 5). The deformation possibly represents a north-west-south-east trending fault, described by Giermann⁷ and Wong *et al.*⁸, but at the northern end the deformed area lies further to the west than these authors have proposed. Widespread tectonic activity seems to be associated with this faulting, because the structural features already described on survey G lie immediately behind and to the east of the fault. The deformed area falls within a broad band of seismic events. Furthermore, on both grids G and H there is good correlation between a broad negative magnetic anomaly and the fault zone, the negative anomaly separating two areas of

TABLE 1 Correlation between velocities measured for Cyprus on land and at sea (sources are indicated by the references)

Outcrop ⁴	Velocity on land (km s ⁻¹)		Velocity at sea (km s ⁻¹)	Formation	Correlation with oceanic crust ^{12,13}
	In formation ¹⁰	Laboratory ¹¹ (0-2 kbar)			
2.9	3.3	2.9-4.3	2.1	Upper sediments	Layer 1
3.7			3.3	Pillow lavas	Layer 2
4.8	5.1	4.9-5.7	4.4, 4.5	Basal group	Layer 2
4.9, 5.1			4.4	Diabase	Layer 3
5.5	6.4	6.6-7.0	5.5	Gabbro	Layer 3
			6.5		

positive anomalies to the north and south. Thus the fault is indicated by reflection, seismicity and magnetic data. If the western boundary of the Turkish plate is delimited by this fault, then the boundary would pass from the west coast of Cyprus, north-west towards Rhodes and the Gulf of Fethiya (Fig. 5), rather than due north to the Gulf of Antalya as drawn by McKenzie^{1,2}.

velocities along X-R, shot from north-east to south-west, are substantially lower than on X. A layer of 5.5 km s⁻¹ is detected when shooting from the north end, whereas a 6.5 km s⁻¹ layer is reached at the southern end. Otherwise, the velocities reverse to give a dipping layer solution as shown in Fig. 7a with velocities of 2.1, 3.3 and 4.4 km s⁻¹. On line Y the topography is extremely irregular so that a large scatter is observed in the arrivals and topographic corrections were necessarily large. A velocity of 2.8 km s⁻¹ is detected from second arrivals only on line Y, (A' in Fig. 6b) which is in fact a multiple of the 3.0 km s⁻¹ refractor by reflection within the upper thin sediment layer. The structure profile is shown in Fig. 7b, with velocities of 1.7, 2.9 and 4.5 km s⁻¹. The last layer was only reached on the reverse line Y-R. A comparison of the Researcher results

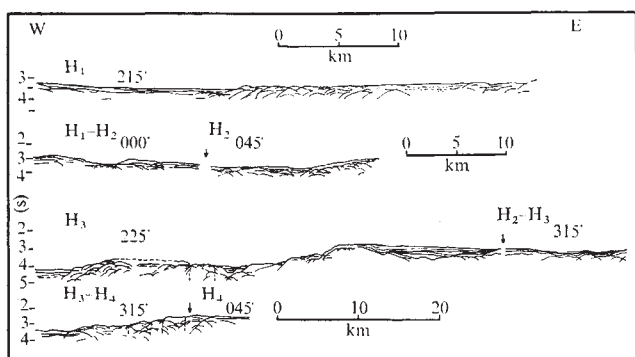


Fig. 4 Line diagrams of sparker reflection profiles, survey H, drawn from west to east. Two-way times are shown.

There have already been refraction experiments on land to measure the seismic velocities at outcrop of rocks in the Troodos complex⁴. In an attempt to correlate these with velocities at sea two short lines X and Y were placed in Famagusta Bay and Morphou Bay. These repeated the seismic experiments of HMS Challenger in 1952 (ref. 6), but over a greater range.

Results are presented in the form of travel-time graphs and structure profiles (Figs 6, 7). The structure on line X seems to dip down towards the south, because apparent

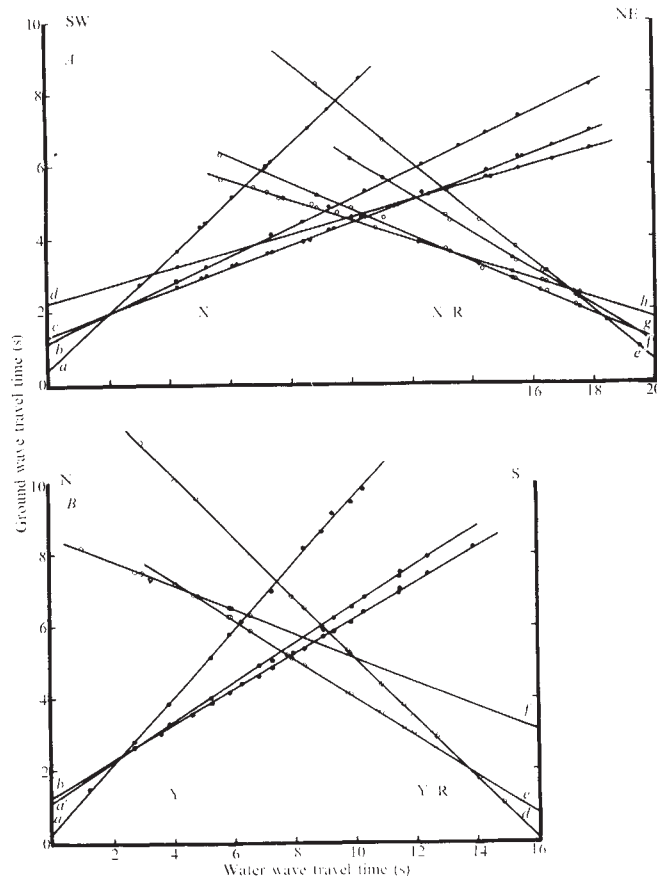


Fig. 6 Travel time graphs for refraction profiles X (6A) and Y (6B). Equations of the times are in the form: intercept time (s) + $x/\text{velocity}$. 6A; a, $0.4 + x/2.0$; b, $1.2 + x/3.8$; c, $1.3 + x/4.9$; d, $2.3 + x/6.5$; e, $0.6 + x/2.2$; f, $1.1 + x/2.9$; g, $1.2 + x/4.1$; h, $1.8 + x/5.5$. a, b, c, d, from profile X; e, f, g, h, from profile X-R, 6B; a, $0.3 + x/1.6$; a', $1.1 + x/2.8$; b, $1.2 + x/3.0$; d, $0.8 + x/1.8$; e, $0.8 + x/2.8$; f, $3.1 + x/4.5$. a, a', b, from profile Y; d, e, f, from profile Y-R.

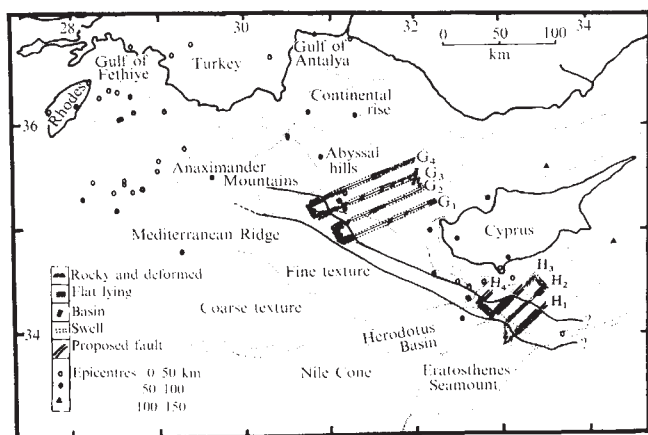


Fig. 5 Correlation of provinces over G and H to show areas of deformation, which correspond to a negative magnetic anomaly, and which possibly constitute a fault zone. Earthquake epicentres are shown. Physiographic provinces after Lort (1972) (ref. 14).

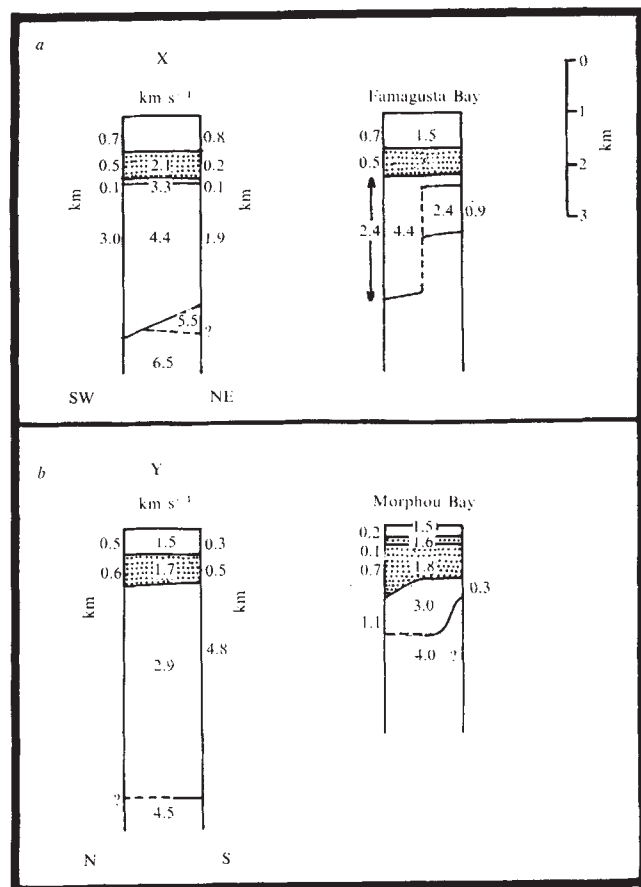


FIG. 7 Comparison of crustal structure profiles near Cyprus obtained by HMS Challenger in 1952 (ref. 6), and MV Researcher in 1971. *a*, line X in Famagusta Bay; *b*, line Y in Morphou Bay. The layer thicknesses are calculated at each end of the profiles as indicated.

with those of Challenger shows a similarity in general structure but deeper penetration on profiles X and Y-R (Fig. 7). A correlation between velocities measured on land and at sea can be seen in Table 1. On line Y, the 4.5 km s⁻¹ layer is tentatively correlated with the pillow lavas which also outcrop on the southern edge of the bay⁹. They are supposedly quite thick as no material of greater velocity is detected even at a range of 22 km. The velocities of 5.5 km s⁻¹ and 6.5 km s⁻¹ probably correspond to layer 3 velocities.

It is apparent from the table that velocities measured in the rocks¹⁰ at outcrop⁴ are generally lower than those obtained in the laboratory¹¹ or at sea. This is undoubtedly an effect of the difference in confining pressure on the rock units in the two environments^{4,10}, and also of the shattered nature of the outcropping units. These results are thus compatible with the widely accepted proposal that Cyprus is a section of oceanic crust¹⁰⁻¹³.

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Flysch-ophiolite successions: polarity indicators in arc and collision-type orogens

ARC systems formed above subduction zones show a characteristic polarity defined by the direction of dip of the seismic Benioff zone along or within which the subducting plate descends. In modern active arcs the polarity is indicated by the relative position of the volcanic arc and trench, by changes in the K₂O/SiO₂ ratio across the volcanic arc¹, and commonly by arc curvature. The polarity of ancient arcs can be determined by variations in the K₂O/SiO₂ ratio in volcanic arcs or their plutonic granodioritic equivalents², by recognition of parallel ophiolite and magmatic belts, or by the presence of paired metamorphic belts³. The polarity in many ancient arc systems, and also in collision-type orogens, is, however, uncertain either because magmatic arcs are poorly developed or because the relative positions of the paired belts have been confused by tectonic movements.

I suggest here that in some arc systems, the polarity can be determined by the predominant younging direction of flysch-ophiolite successions. In collision-type orogens, the polarity is indicated either by the younging direction of the flysch or by the direction from which the flysch is overthrust.

Recent work on Barbados⁴ provides evidence of the mechanism of underthrusting and deformation within pelagic sediments and turbidites deposited on the ocean floor and carried into the subduction zone. As these flysch-type sediments approach the subduction zone a thrust plane inclined towards the zone develops, cutting the sediments at a low angle (Fig. 1*a*). Continued subduction and underthrusting results in emplacement of the sediments overlying the thrust plane in a tectonic wedge above the subduction zone. Younger sediments deposited on the subducting plate further away from the plate boundary are in turn cut by a further thrust plane as they near the subduction zone, and are underthrust by still younger sediments. A tectonic pile of sedimentary wedges results, in which the overall age of the flysch increases towards the over-riding plate. Within each tectonic wedge, however, the age of the sediments decreases in the same direction (Fig. 1*b*).

Major belts consisting largely of late Mesozoic or Cainozoic flysch, deformed in a style similar to that of Barbados, occur in some other active or recently active arc systems, for example the Sunda-Burma arc⁵, southern Alaska⁶ and north-west Borneo^{7,8}. Within these belts part of the ophiolite sequence serpentine-gabbro-dolerite-pillow lavas, overlain by radiolarian cherts, is locally present beneath the flysch, forming a 'eugeosynclinal' association of lithologies. The ophiolite sequences can be interpreted as part of the oceanic crust from the subducting plate, scraped up and emplaced together with the overlying flysch above successive thrust planes (Fig. 1*c*).

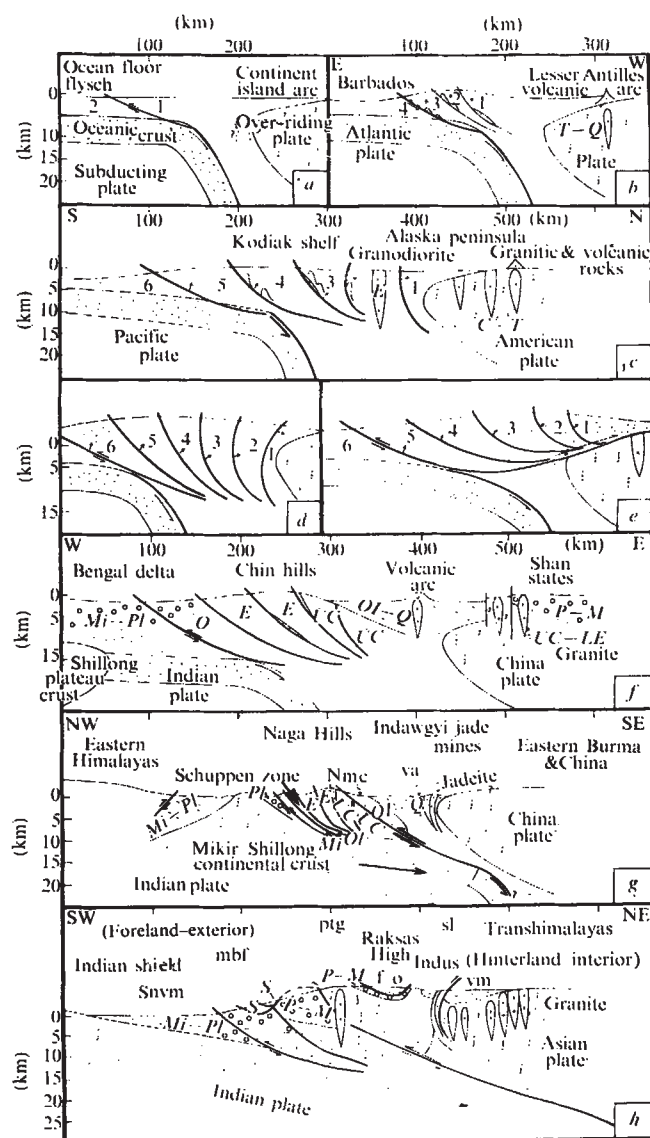


FIG. 1 Schematic simplified cross sections through Cretaceous arc systems and collision-type orogens. Locations of cross sections shown in Fig. 2. *a*, Initial underthrusting during start of subduction (no known example); *b*, Barbados (modified from Westbrook *et al.*⁴); *c*, southern Alaska showing plutons intruding flysch (modified from Moore⁹ and (Martin)¹⁰); *d*, overturning of flysch due to progressive steepening of thrust units (no Cretaceous example known); *e*, tectonic emplacement of flysch on overriding continental margin (no Cretaceous example known); *f*, Chin Hills, Upper Burma (Modified from Brunnschweiler¹⁴ and others (references in Mitchell and McKerrow)¹⁰); *g*, Naga Hills, Upper Burma (Modified from Brunnschweiler¹⁴); *Nmc*, Naga metamorphic complex; *va*, volcanic arc; *h*, Kumaon Himalayas. (Modified from Gansser)¹⁶. Alpine terminology in parentheses; *Snvm*, Siwaliks non-volcanic molasse; *mbf*, main boundary fault; *ptg*, post-tectonic gneiss; *f-o*, flysch and ophiolites; *sl*, suture line; *vm*, volcanic molasse. Possible sequences of events in one arc system: *a* → *b* → *c* (or *d*) → *f* → *g*; *a* → *e*; *a* → *b* → *f* → *h* if flysch absent throughout subduction. ~, fluviatile and molasse facies; open circles, shelf or delta top facies; heavy dots, ophiolites; faint dots, flysch; *S*, continental crust of subducting plate; *f*, continental or island arc crust of over-riding plate. 1, 2, 3 . . . , successively younger flysch units. Arrows indicate younging direction. *P*, Palaeozoic; *M*, Mesozoic; *C*, Cretaceous; *T*, Tertiary; *E*, Eocene; *O*, Oligocene; *Mi*, Miocene; *Pl*, Pliocene; *Q*, Quaternary; *L*, lower; *U*, upper.

Within some flysch-ophiolite belts granodioritic or granitic intrusions slightly younger than the flysch are present. An example occurs in southern Alaska, where early Tertiary

granodiorites intrude the older part of a belt of tectonically repeated late Mesozoic and Cainozoic flysch with minor ophiolites. Here the flysch youngs mostly northwards within each tectonic unit. These 'post-tectonic' plutons represent magmatic arc activity above a Benioff zone which either increased its inclination or migrated oceanwards relative to its position during the Cretaceous⁹ (Fig. 1*c*).

An example of a deformed flysch-ophiolite succession in an ancient orogen is provided by the Lower Palaeozoic succession of the Southern Uplands in Scotland, where imbricate successions of north-westward-dipping turbidites overlie pillow lavas and cherts¹⁰. In Ireland, in part of the western continuation of the Southern Uplands the turbidites are overturned to the north (A. E. Phillips, personal communication). This overturning possibly resulted from progressive steepening of the already emplaced tectonic wedges during underthrusting by younger sediments (Fig. 1*d*).

In the southern Alaska flysch belt there is evidence that the thrust planes converge downwards towards a zone inclined beneath the continent⁹. It is possible, however, that in some other arcs the thrusts could converge in a plane of decollement, convex downwards, resulting in overthrusting of the flysch belt onto the continent (Fig. 1*e*). A possible example of this is found in the Patagonian Andes, where Mesozoic flysch is thrust eastwards over a miogeosynclinal succession and volcanic belt¹¹.

The flysch successions considered can be interpreted as submarine delta fan deposits which accumulated mostly on the ocean floor and perhaps partly in a trench. In some arc systems in which little flysch reaches the ocean floor, for example the Ryukyu Arc, a thick succession with a seismic velocity typical of sediments is present on the arc side of the trench¹². This succession possibly consists largely of pelagic sediments tectonically emplaced in a similar way to the flysch, although analogous thick belts of tectonically repeated pelagic successions are rare in ancient orogens.

In the Cainozoic arc systems considered, the polarity is invariably indicated by the predominant younging direction of the flysch within each thrust slice, which is towards the over-riding plate. The flysch is commonly tightly folded and overturned adjacent to, and particularly above, thrust planes. But where the thrust planes themselves are overturned as a result of oversteepening the flysch still youngs towards the over-riding plate.

Evidence for successive underthrusting of flysch-ophiolite wedges in arc systems suggests that a similar process could affect continental crust and overlying shelf sediments which are underthrust prior to and during continental collision.

In the Cainozoic Burman arc, which developed above an eastward-dipping Benioff zone, delta top sediments have been underthrust beneath the flysch-ophiolite belt of the Chin Hills^{13,14} (Fig. 1*f*). Further north, continental crust of the Indian Shield, exposed in the Mikir Hills, is underthrusting

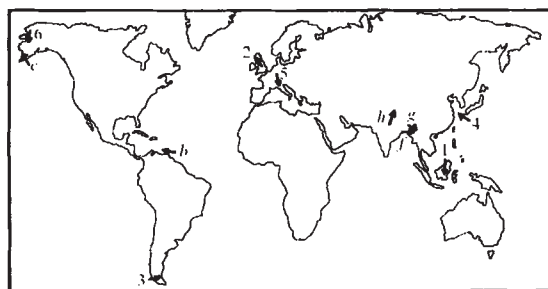


FIG. 2 Locations of cross-sections shown in Fig. 1 and of localities referred to in text. Arrow points towards known or inferred subduction direction. *b*, Barbados; *c*, southern Alaska; *f*, Chin Hills; *g*, Naga Hills; *h*, Kumaon Himalayas; 1, north-west Borneo; 2, Southern Uplands; 3, Patagonian Andes; 4, Ryukyu Arc; 5, Swiss Alps; 6, northern Alaska.

deformed flysch and ophiolites of the Naga Hills (Fig. 1g)¹⁵. Shelf sediments of the Indian plate are involved in thrusting in the west, and in the east the Naga metamorphic complex is thrust westwards over the flysch¹⁴. The metamorphic complex can most simply be explained as an upthrust slice of the continental Indian plate. This requires that the continental crust of the Indian plate has overridden the original subduction zone, the position of which is shown in Fig. 1f, and underthrust the continental part of the China plate (Fig. 1g). The belt of jadeite-albite dykes and glaucophane schists at Indawgyi Lake can then be interpreted as oceanic crustal rocks dragged downwards along or above the Benioff zone, and subsequently thrust upwards together with the metamorphic complex.

Although flysch is largely absent in the Kumaon Himalayas¹⁶, the Himalayan structural setting broadly resembles that of the Naga Hills, and can be explained by northward subduction followed by underthrusting of the Indian Shield towards and beneath Tibet¹⁷. Metamorphic rocks and shelf sediments within thrust slices in the Kumaon Himalayas¹⁶ represent tectonic wedges of the Indian Shield and overlying shelf deposits, and the Rakasas High, south of the Indus Suture, is possibly a tectonic slice of the Indian Shield analogous to the Naga metamorphic complex in Burma (Fig. 1h). 'Post-tectonic' granites in the Himalayas may have resulted from the final stages of subduction along the original Benioff zone, the inclination of which approached the vertical as a result of the slowing down and stopping of subduction.

In the Naga Hills collision-type orogen, as in arc systems, the polarity is indicated by the younging direction of the flysch. Where, however, the flysch either dips at low angles, or shows no predominant inclination, as in the Kumaon Himalayas, polarity is indicated by the direction of overthrusting, which is directed towards the subducting plate¹⁷.

In the complex collision belts of the European Alps, overthrust slices of metamorphic rocks and shelf sediments, analogous to those present in the Himalayas and Naga Hills, can be interpreted as tectonic wedges of the underthrusting continent on the subducting plate, rather than crustal flakes from the over-riding plate¹⁸. In the eastern Alps the northward direction of thrusting of the ocean floor rocks suggests that the polarity, or direction of underthrusting, was to the south rather than to the north as required by the flaking model¹⁸, although it is possible that, at depth, the Benioff zone dipped to the north. The Alps are complicated by major gravity slides, but the relative positions of structural units resemble those of similar units in the Himalaya¹⁶ or Naga Hills collision belts (Fig. 1g and h). The contrast between the predominant metamorphic thrust units in the Himalayas, and the flysch-ophiolite successions in the Naga Hills and many of the Alpine chains, indicates that delta fan deposits were absent in the Tethys ocean north of India during the Cretaceous and early Tertiary.

Structures similar to those formed in a continent-continent collision could result from the collision of a continent on a subducting plate with an island arc on the over-riding plate. In northern Alaska northward overthrusting of the Brooks Range miogeosynclinal succession by similar shelf facies, and by Jurassic flysch and ophiolites of the Koyukuk geosyncline to the south^{19,20}, possibly resulted from attempted southward subduction of the miogeosyncline beneath the flysch. The Tintina Trough could then represent the suture line, analogous to the Indus Suture, between the Koyukuk flysch to the north, and a late Jurassic subduction-related island arc complex in the Ruby geanticline on the over-riding plate to the south.

Flysch-ophiolite successions are present in many Phanerozoic and Late Precambrian orogens, some of which can be interpreted as arc systems and others as collision belts. The younging direction of the flysch in both types of orogen,

and the direction from which the flysch was thrust in collision-type orogens, can indicate the subduction direction and hence the polarity of parts of these orogens. Evidence for polarity, essential to interpretations of the orogens, is of increasing use in the search for mineral deposits related to magmatic arcs and of indirect use in petroleum prospecting within and adjacent to the younger orogens.

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Hot spot in France?

THE hypothesis of hot spots is central to many discussions about volcanism; it was first put forward by Wilson¹ and has just been reemphasised by Morgan² who proposes that a hot spot corresponds to the top of a plume rising from the deep mantle³. As a plate drifts above it, volcanic activity takes place, giving rise to a linear chain structure with a uniform progression of ages from the active head in a direction defined by the reversed absolute drift velocity of the plate.

The best studied example is the Hawaiian volcanic island chain. More recently, Wilson and Burke⁴ have proposed a hot spot origin for many volcanic structures on ocean floors and on continents. So the concept of hot spots cannot always correspond to a deep mantle plume but may be related to a thermal inhomogeneity located only in the asthenosphere. A uniform progression of ages along one azimuth is still expected, however, unless the plate involved is stationary relative to the mantle.

Could volcanism in France be associated with a hot spot? We do not give a clear-cut answer to this question here; rather we emphasise that no simple cinematic linear model is consistent with the ages of the volcanic provinces (Fig. 1) associated with the Massif Central. Schematically, the pattern has three branches diverging from a centre of volcanism, 20 Myr old, the Cantal; the extreme end of each branch is characterised by recent activity. Similar patterns have been

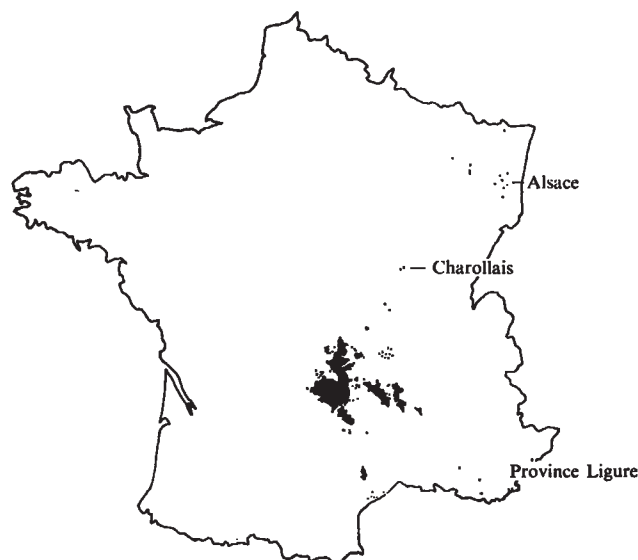


FIG. 1 Volcanism in France.

analysed by Dewey⁵. On a wider scale, however, some linear age progression is evident.

Between 20 and 13 Myr ago volcanic activity was not very prevalent. The formation of the Cantal caldera was probably connected with abundant pyroclastic emissions between 13 and 7 Myr ago. Important basaltic or trachyandesitic lava flows also took place and phonolites appeared around 8 Myr ago (ref. 14). We follow this, first, along the northern branch: the Mont-Dore started with a pyroclastic phase 8 Myr ago and true rhyolitic outflows only began 2.2 Myr ago; the activity stopped 0.4 Myr ago (ref. 15). The corresponding rectangle in Fig. 3 illustrates the geographical spread to the north and the age interval involved. Further north the Chaîne des Puys started 0.15 Myr ago (R.B., H.B., and A. Rudel, personal communication) and was still active in 80 B.C. (ref. 16). As radiogenic dating is not available for the Cézallier, the corresponding rectangle on Fig. 3 is not well determined. From its morphology, this province seems to be older than Mont-Dore.

Simultaneously, the southern branch started 13 Myr ago in Cantal, where the overall activity stopped about 3 Myr ago (ref. 12). Next came the Aubrac and then several well aligned provinces¹⁷⁻¹⁹ down to the coast near Agde, where the most recent outcrops are dated 0.7 Myr. (ref. 9). Younger outflows further south may have been buried on the crumbled floor of the Mediterranean Sea.

The third branch spreads to the south-east and is made up of two distinct echelons: the Velay-Coiron alignment (which stopped being active at 4 Myr (ref. 20)) and further south of the Devès-Ardèche, in which province the most recent basaltic flows took place about 12,000 yr ago, (ref. 21; Fig. 3).

Before describing the age and space structure of the Massif Central in more details, we note the existence of two other distinct volcanic phenomena in France (Fig. 1). In the Ligurian province, the andesitic rocks dated 26 to 29 Myr (ref. 6) originated at the end of the Oligocene and are probably associated with a past subduction zone⁷. Rift volcanism is well known on the sides of the Rhine graben (between 80 and 0.4 Myr (refs 8 and 9)). There are other graben formations to the south-east of the Rhine valley forming an *en échelons* pattern (Fig. 1): the Charollais graben also shows Eocene volcanic outcrops dating from 60 to 30 Myr ago (ref. 10); no volcanism is known in the Dombes graben. The Roanne-Montbrison graben is also volcanic, and finally the Limagne has volcanic outcrops both on its central axis (22 to 3 Myr (ref. 11)) and along its granito-metamorphic sides.

Thus subduction, on the one hand, and graben formation in connection with the alpine tectonics involving a possible shearing or extension between the Hercynian platform and the Alps, on the other, are able to explain the volcanic activity described, the total volume of which is rather modest by comparison with the Massif Central.

The spreading of volcanic activity along the three branches of the Massif Central can be analysed on the basis of Fig. 2. An initial outflow took place 21 Myr ago¹² at the limit between the north of Cantal and the Cézallier massif. This location lies at the north-west end of an Hercynian horst containing the only known peridotite residuals of the region¹³, witness of a very old (Cambrian?) suture. We take it as the geometric origin of volcanism, noting, however, that other Lower Miocene, mainly basaltic, outcrops have been dated all around the Cantal massif in direct contact with the crystalline basement.

We do not discuss here the important question of the chemical evolution of magmatism with time or structural location. The facts we stress are the following: (1) As far as volume of emission is concerned, the provinces associated with the Massif Central are the dominant feature of volcanism in France. (2) Activity has spread along three arms around

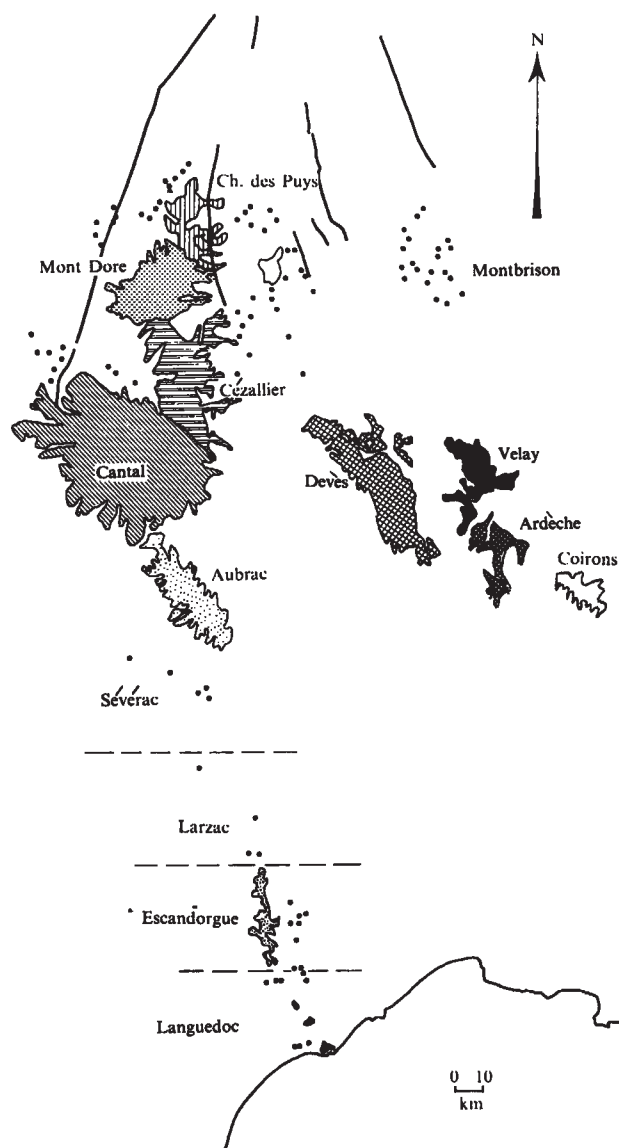


FIG. 2 Volcanism of Massif Central, Causses, Escandorgue and Languedoc. Each volcanic district is represented with a specific graphical design. Each of them is represented by the same specific graphical design in Fig. 3.

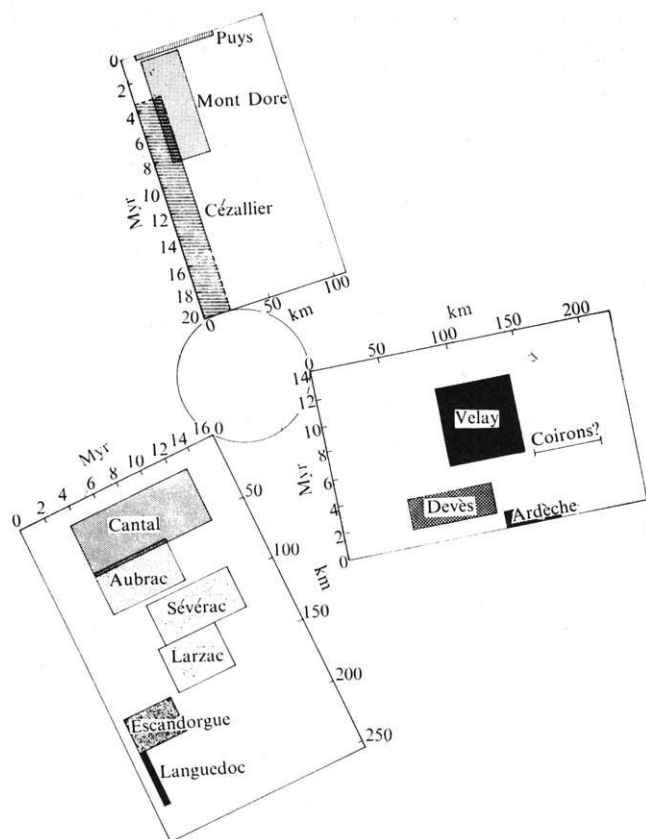


FIG. 3 The great circle represents the situation of the starting point of French volcanism 20 Myr ago. Each of the three rectangles is respectively a diagram of the three branches along which volcanism spreads. Inside the big rectangles, small ones are measured in time (Myr) representing activity within a province (duration of volcanic activity) and in km (distance of the province (proximal and distal edges) from the starting point).

the region of initial volcanism in Cantal dated 20 Myr. If this spreading is assumed to have started 13 Myr ago, the average spreading velocity can be estimated from Fig. 3 to be 0.6, 1.7 and 1.0 cm yr⁻¹ along the northern, southern and south-eastern branches respectively. Although the accuracy of these figures is poor, the difference between them is real. (3) The volume of volcanic products emitted decreases approximately exponentially from Cantal to the extreme end of the three branches. For example the Cantal massif is about 10 times as big as the Mont-Dore, which is in turn about 10 times bigger than the Chaîne des Puys.

So we conclude that the age and space structure of the Massif Central does not exclude or prove the presence of hot spot under the European lithosphere in this area. It certainly does not yield a quantitative knowledge of the drift velocity of the European plate with respect to the asthenosphere beneath it.

Returning to the general distribution of volcanic centres in France, the age of initiation of rift volcanism progresses quite uniformly as one moves from the Rhine graben (80 Myr) to the Charollais (60 Myr), the Limagne (22 Myr) and finally the southern branch of the Massif Central, which lies along this general trend. It is tempting to connect this with a hot spot. The spreading velocity of the latter would be non-uniform, averaging about 1 cm yr⁻¹. Such a proposal immediately raises questions: What is the relationship between volcanic activity and tectonism? Why does the magmatic activity last for so long after the initial phase? What causes the occurrence of a paroxysm in the activity around 13 Myr connected with the initiation of a three-branches pattern? Finally, there are difficulties in reconciling this overall

north-south trend with the suggestions of Duncan *et al.*²² of an east-west progression of volcanic activity from Poland to the Eiffel and a south-north migration from Scotland to Ireland. Dating and magmatic studies on a large scale at the periphery of the Alps is vital if a comprehensive model of volcanism on the European plate is to be built up.

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Glacial and postglacial sedimentation in the Sea of the Hebrides

SHALLOW seismic profiling, sediment coring and drilling from an anchored ship have provided new information about sedimentation in the Sea of the Hebrides. Some initial results are summarized here.

On shallow seismic profiles over the inner continental shelf (cast of 08°00'W, Fig. 1) a principal reflector, coincident with a stratigraphic unconformity, is interpreted as a rockhead reflection. This surface is obviously glaciated¹, overdeepened trenches are common, and where it crops out it is smoothed and striated². The layer above this reflector is, therefore, interpreted as Quaternary sediment. Its thickness has been calculated assuming a seismic velocity of 1.8 km s⁻¹ and isopachytes are shown in Fig. 1. Cores and boreholes through this layer have recovered the following lithologies.

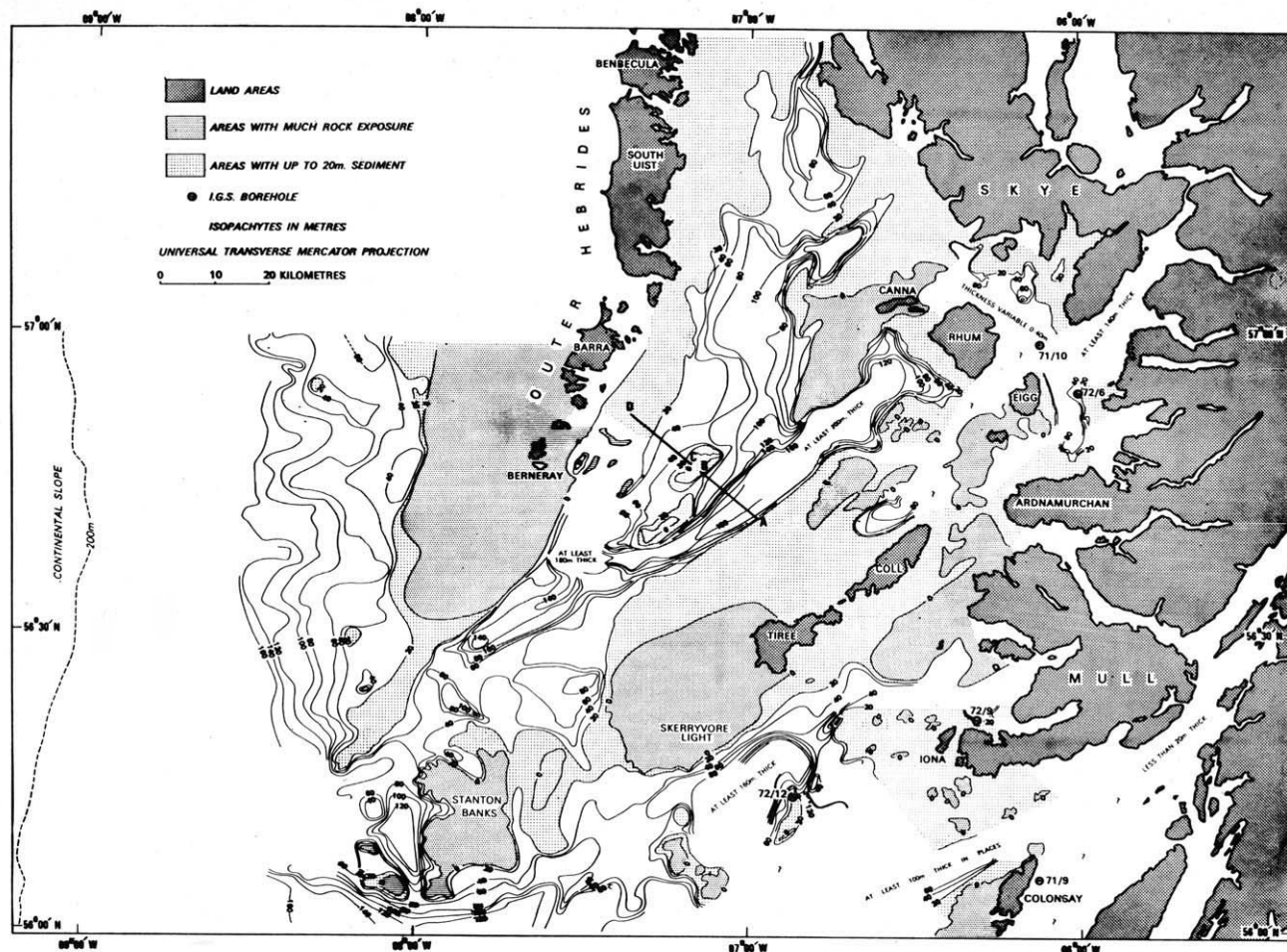


Fig. 1 Isopachytes on Quaternary sediment.

Formation 1. Till (Fig. 2) constitutes only a small proportion of the total sediment encountered in the boreholes. Reflections from the surface of moraine hummocks are seen on some seismic profiles (Fig. 3) but more commonly the profiles fail to record a boundary between Formations 1 and 2. This may be due to lack of velocity contrast between the two or to an insignificant thickness of Formation 1.

Formation 2. These firm, poorly sorted sandy muds³ (Fig. 2) contain variable amounts of granule and pebble grade lithic fragments⁴. Benthonic foraminifera are sparse but there is a significant dinoflagellate cyst population.

Lithology (compare with refs 5 and 6), stratigraphical position beneath late glacial sediments of Formation 3 and distribution on the courses of the major ice streams together suggest that Formation 2 was deposited in close association with ice. In spite of the firmness of the sediment and the presence of gravel grade material, the consistently distinctive marine dinoflagellate cyst assemblages indicate that this formation comprises marine sediment and not till. The nature of the 'till' in borehole 72/12 is not understood; it may be a submarine 'flow till'⁵ rather than a true till.

Formation 3. A pilot study of this formation in borehole 71/9 shows that the sediments are poorly sorted sandy muds³ similar to Formation 2. They are, however, softer and contain medium sand to pebble grade fragments only rarely, and then at levels coinciding with cold water faunal assemblages.

Study of foraminifera and dinoflagellate cysts has shown that the Formation 3 sequence can be divided into three palaeoclimatic zones¹. From -30 m to -28 m the foraminiferal population is dominated by *Ammonia batavus* (Hofker), a warm, shallow water species. A rich assemblage of dinoflagellate cysts indicates a similar environment. Between -26 m and -16 m a cold water assemblage of foraminifera,

dominated by *Elphidium clavatum* Cushman, coincides with poor recovery of dinoflagellate cysts. From -14 m upwards cold water foraminifera decline in importance. The dinoflagellate cysts from -10 m to the surface are modern in aspect.

The disseminated organic matter contains significant amounts of reworked Mesozoic material. The least contaminated sample was at -6 m; of the organic-walled microplankton only 14.7% were Mesozoic forms and amorphous organic matter appeared fresh. A date of $9,961 \pm 250$ BP was obtained from the disseminated organic matter at this depth (Scottish Reactor Research Centre 117). This has been taken as a maximum age which reduces to 8,680 BP on the assumption that 14.7% of the carbon is old. Assuming continuous deposition it suggests that the older 'warm' water fauna belongs to the Alleröd interstadial, the 'cold' water fauna to the period of the Loch Lomond readvance stage and the younger 'warm' water fauna to the final climatic amelioration.

Formation 3 sediments are considered to have been deposited at some distance from the ice front (compare with the 'periglacial marine' sediments of southern Alaska⁷). During deposition of the sequence in borehole 71/9, ice, when present, lay behind the present coastline; a plentiful supply of sediment could be expected both from marine erosion of the sea floor and coastline and from fluvio-glacial sources. This would account for the high sedimentation rate implied by the date. A similar succession has been described on the east coast of Scotland (J. D. Peacock, to be published).

The poor recovery of dinoflagellate cysts between -26 m and -16 m may be attributed to a continuously high level of suspended matter (produced by the Loch Lomond readvance stage) and not to low water temperature. The sus-

pended matter would adversely affect the thecate dinoflagellate population and add a dilution factor resulting from high sedimentation rates⁸. In contrast the significant populations of Formation 2 are inferred to have lived in clear, cold water adjacent to floating ice. Potential suspended matter remained frozen into the ice to be released intermittently, perhaps by seasonal melting, to form Formation 2 deposits.

Formation 4. These sediments form a thin layer (0.05 m to 2.0 m) on all older formations. Their character can be related to bathymetry and situation with respect to the prevailing south-westerly swell. They are interpreted as modern sediments.

Calibration of shallow seismic profiles by the boreholes suggests that Formations 2 and 3 each produce a characteristic seismic texture which can be used to determine their distribution. Formation 3 typically has closely spaced, horizontal reflectors (Fig. 3) which fade laterally and which cannot be correlated with any visible structure in the boreholes. A second characteristic texture, interpreted as Formation 2, has widely spaced reflectors (Fig. 3). Only borehole 72/12 has passed through this unit but if the interpretation is correct then most of the thick sediments west of Colonsay are of Formation 2, as are those in the trench northwest of Coll (Figs. 1 and 3).

West of Colonsay Formation 3 sediments fill shallow depressions on the surface of Formation 2. In contrast, east of Colonsay, in an area protected from the prevailing south-westerly swell, nearly 30 m of Formation 3 sediments have been deposited and preserved at a topographically higher level (compare boreholes 71/9 and 72/12, Fig. 2). Formation 3 sediments also occur in the trench north-west of Coll (Fig. 3) and in the glacial trenches around the Inner Hebridean islands. Considerable variations in their thickness, however, suggest deposition (or subsequent erosion) under the control of a complex system of currents.

On the outer continental shelf the sea floor is relatively flat but the rockhead reflection drops evenly westwards¹. There are no trenches overdeepened by glacial action on rockhead and so the possibility that Tertiary sediments lie above it cannot be excluded.

The two seismic textures on profiles west of Colonsay are also present here. Short (0.8 m) cores taken at the outcrop of the lower unit penetrated a thin (0.1 to 0.5 m) cover of Formation 1 sands and gravels to recover Formation 2 sediments.

Evidence from the land shows that a major ice sheet originating on the mainland moved westwards to extend across the outer shelf. In contrast to this evidence, overdeepened trenches off shore trend along north-east to south-west structures¹. It has been inferred¹ that initially the area was in-

vaded by south-westerly moving ice streams from local high ground, but that during maximum glaciation the westerly moving ice sheet crossed the whole area leaving striae and erratics on areas which are now land. This sequence was reversed during deglaciation.

Deposition of the sediments would have been controlled by the nature of the ice and the manner of its retreat, in particular its relation to changes in sea level. A complex history of sedimentation is thus indicated and the reconnaissance nature of the evidence presented here allows only outline conclusions to be drawn.

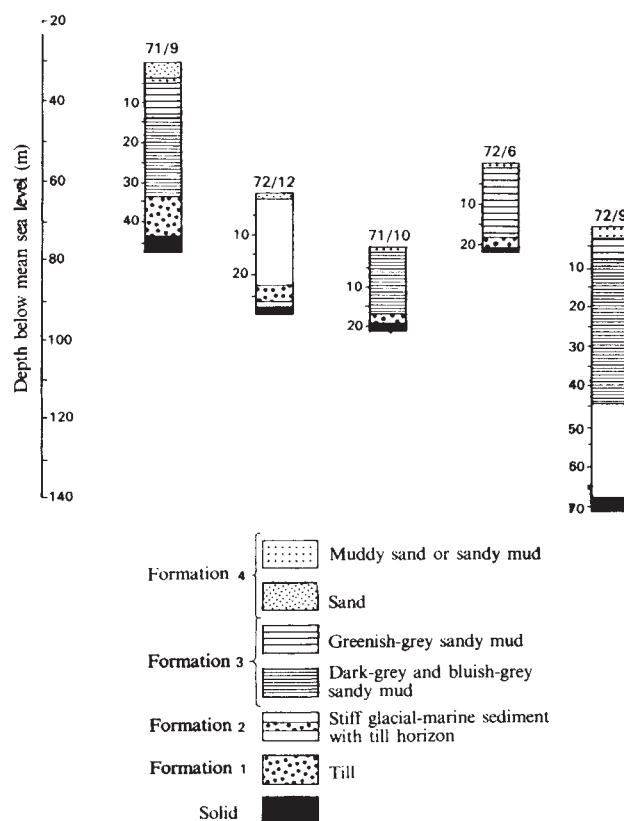


FIG. 2 Boreholes through Quaternary sediment.

It is suggested that the four formations were deposited diachronously by the retreating ice of the last (Devensian) ice sheet. The Formation 2 sediments on the outer continental shelf (Fig. 1) were deposited by the main ice sheet, and those west of Colonsay and north-west of Coll (Figs 1 and 3) by major, south-westerly moving ice streams, probably at a more advanced stage in the retreat. Their thickness reflects

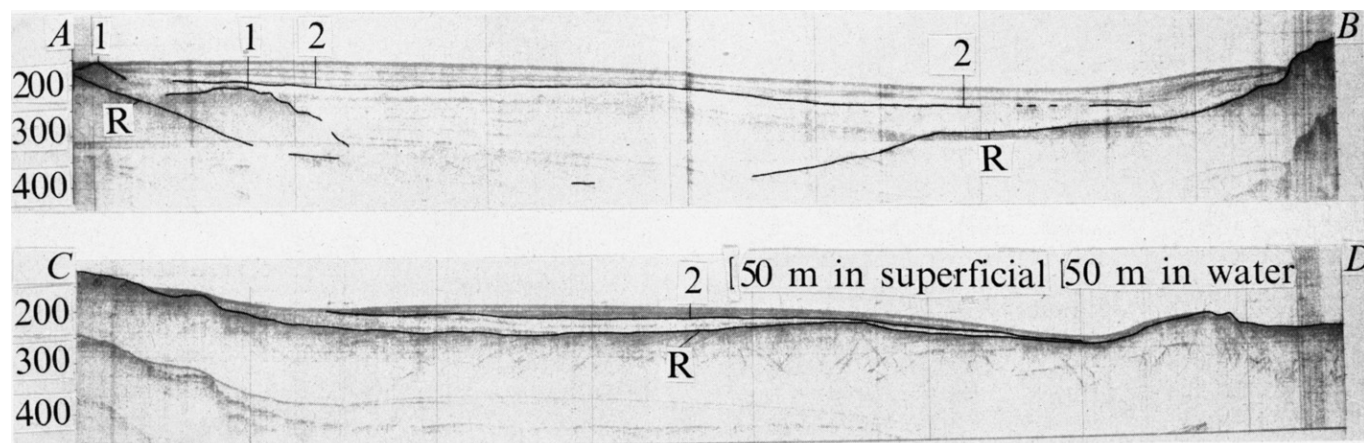


FIG. 3 Shallow seismic sections A-B and C-D. For locations see Fig. 1. Vertical scale in ms, two-way travel times below sea floor. R, rockhead; 1, surface of Formation 1; 2, surface of Formation 2.

the size and activity of the parent glaciers. Formation 3 sediments were deposited at a distance from the ice. When the supply of Formation 3 sediment became exhausted and sea level readjusted to its present position the deposits of Formation 4 were formed.

This study is being undertaken as part of the research programme of the Institute of Geological Sciences.

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Carbon fibres with large breaking strain

It has been predicted¹ that carbon fibre with a Young's modulus near 200 GN m⁻² could be prepared from acrylic precursors to yield elastic breaking strains of 2 to 3% with the proviso that an internally clean precursor was selected, judiciously processed and then surface treated to remove the more serious surface flaws. The prediction was made on the basis of gross internal and surface flaws controlling the strength characteristics of acrylic-based carbon fibres, at least in the region of carbonisation temperatures 1,000 to 1,300° C and in the associated modulus range 140 to 240 GN m⁻².

Fibres with such properties have now been prepared in experimental quantities. The breaking strain of carbonised (1,000° C) acrylics with no subsequent surface treatment is limited to about 1.0% in the modulus range 140 to 210 GN

TABLE 1 Single fibre properties of as-processed fibre; gauge length 23 mm

Cross-sectional area (10 ⁻¹² m ²)		Strength, (GN m ⁻²)		Young's modulus (GN m ⁻²)		Breaking strain (%)	
Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
48.1	5.9	3.17	0.30	207	10	1.53	0.20

m⁻² (ref. 2) and fibre processed to much higher temperatures inevitably yields lower breaking strain^{3,4}. Apart from an isolated report of a few ultra-high strength carbon fibres from Japan⁵, commercial fibres are being offered⁶ which have been surface-treated to give mean breaking strains just above 1.3%.

The acrylic previously shown to have a low internal flaw concentration of 10 flaws per metre (ref. 1) was processed through a batch oxidation, continuous carbonisation route to 1,100° C. The resultant fibre had single fibre properties quoted in Table 1.

TABLE 2 Frequency of fracture initiation by internal flaws¹².

Gauge length (mm)	Internal flaw fracture frequency $F_{int}/(F_{int} + F_{sur})$	Precursor internal flaw prediction $(1 - P)$
10	0.13	0.11
23	0.31	0.33
50	0.30	0.45

Because of the excellent control of processing parameters during preparation the Young's modulus was acceptable and the breaking strain already high. These fibres were submitted to an improvement of previously used surface treatments^{1,3,7,8} to modify the surface flaws normally operating. Treated fibres were then tested in tension by a rigorously controlled testing technique at gauge lengths of 10, 23 and 50 mm; at least 25 fibres were randomly selected for each gauge length. In addition a microscopic loop test⁹⁻¹¹ was carried out to estimate breaking strain at the very short effective gauge length of 0.1 mm. Because the loop test is particularly insensitive to well-spaced but severe flaws it provides some idea of the intrinsic breaking strain between severe stress-raising discontinuities.

Cross-sectional areas of the dog-bone-shaped fibres (Fig. 1) were determined by planimeter measurement of photographic enlargements of collected fractured ends at $\times 2,500$. This

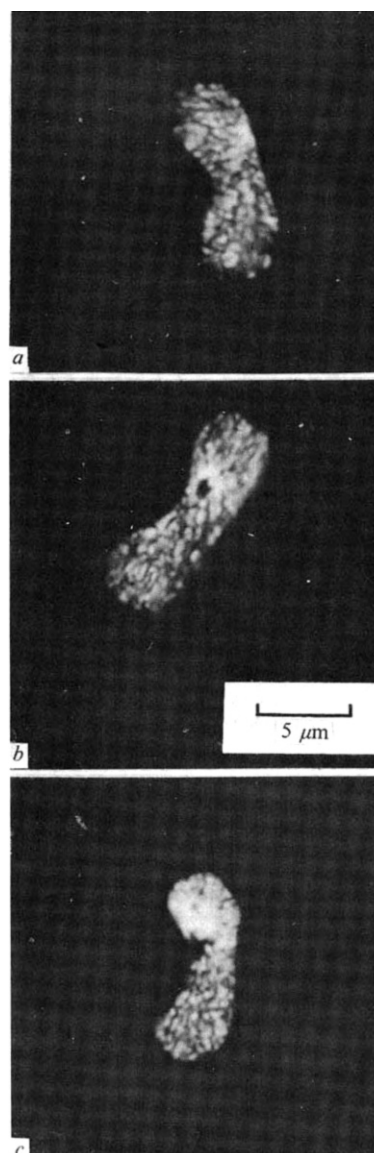


FIG. 1 Fracture surfaces of fibres with high breaking strain broken in tension ($L_g = 50$ mm), showing fracture initiated at: a, modified surface flaw ($\epsilon = 2.21\%$); b, internal flaw ($\epsilon = 1.78\%$); c, residual severe surface flaw ($\epsilon = 1.1\%$).

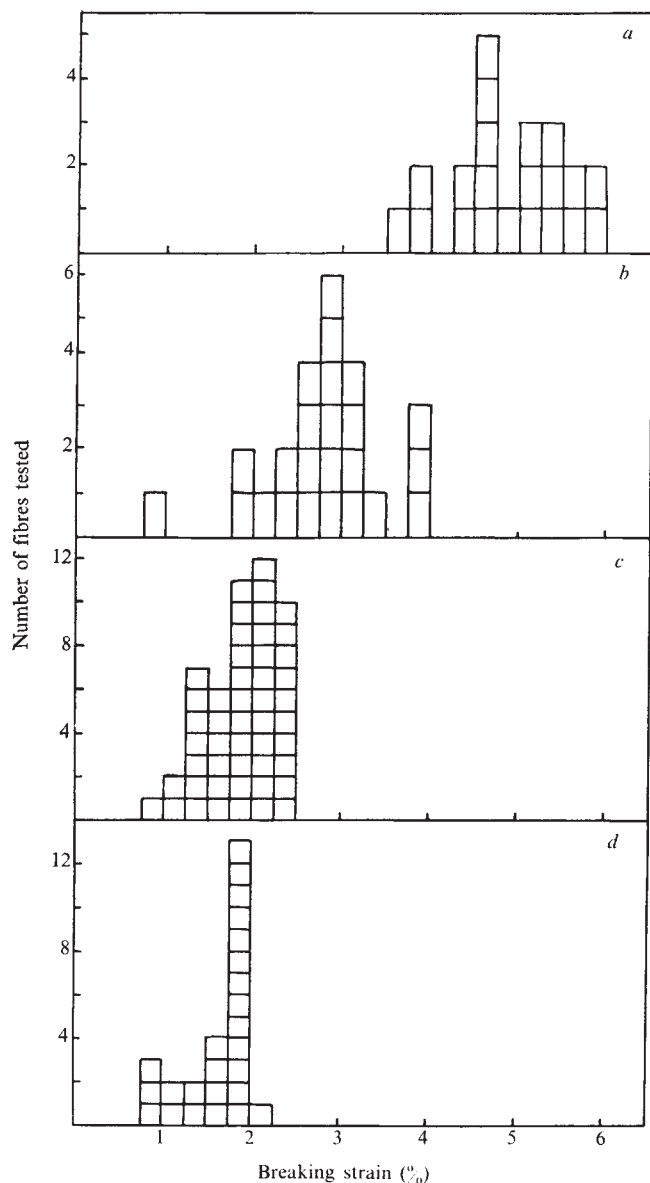


FIG. 2 Breaking strain distributions for various gauge lengths. *a*, Loop; *b*, $L_g = 10$ mm; *c*, $L_g = 23$ mm; *d*, $L_g = 50$ mm.

method has been cross checked for round fibres against normal optical measurement of fibre diameter. Although the probable error is still large at $\pm 7\%$, at least the area is determined at the point of fracture so it is likely to be more correct for fibre strength than for modulus (for which a mean cross-sectional area along the gauge length is required). The Young's modulus value has been verified by alternatively testing the fibres in an epoxide resin composite using standard composite techniques. Breaking strain determinations however, remain independent of fibre cross-sectional area and are favoured in the present discussion. Since the fibres behave essentially elastically to the point of failure, values of breaking strain adequately reflect the strength characteristics.

Examination of fractured ends of fibre, previously carried out by scanning electron microscopy^{1,3,7,12}, was carried out by high quality optical microscopy. The fracture pattern invariably indicated the fracture initiation source (Fig. 1). From fractography studies the frequency of fracture initiation by internal flaws¹ could be elucidated as well as breaking strain distribution associated with those internal flaws.

Breaking strain distribution data are given in Fig. 2 for various gauge lengths. There is a strong gauge length effect but mean breaking strains in excess of 2.0% are achieved at

gauge lengths less than 23 mm. Such strains will, it is believed, become operative in normal tensile loading of well bonded CFRP composites. It is also strikingly obvious that there has been a significant increase in the loop breaking strain ($\bar{\epsilon} = 4.9\%$) over the previously reported values of $\epsilon = 2.8\%$ (ref. 1) and $\bar{\epsilon} = 3.2\%$ (ref. 11). A typical loop at an apparent strain of 5.0% is shown in Fig. 3. At these high strains the loop was slightly distorted into a spiral. This can provide a significant underestimation of loop diameter in normal microscopic observation, correction for which was made by additionally measuring the height difference between sides of the loop at the point where loop diameter is measured.

Spiral distortion also introduces a deviation from elastic behaviour and $\epsilon = r/R$ (ref. 11); the loop strains are probably overestimated therefore but it can be safely assumed they are well in excess of 3.0%. This conclusion is supported by the significantly high proportion (33%) of fibres which had breaking strains in excess of 3.0% in the tensile tests at 10 mm gauge length (Fig. 2).

In normal tensile tests of this and other samples from the same acrylic precursor a significant number of fractures were initiated at internal flaws to provide the distribution of Fig. 4. These data were not available in previous studies¹ to adequately predict the gauge length effect of internal flaws. It was previously assumed that when internal flaws caused

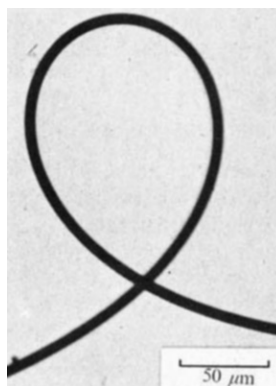


FIG. 3 Fibre sustaining a strain of 5.0% in loop test.

fracture the strength would be reduced on average to 0.33 of the intrinsic strength between flaws (loop test). It can now be seen for this particular acrylic-based fibre that the internal flaws are not very severe and operate in a comparatively narrow distribution about a mean breaking strain of 1.71% (mean strength 3.5 GN m^{-2}). If these internal flaws

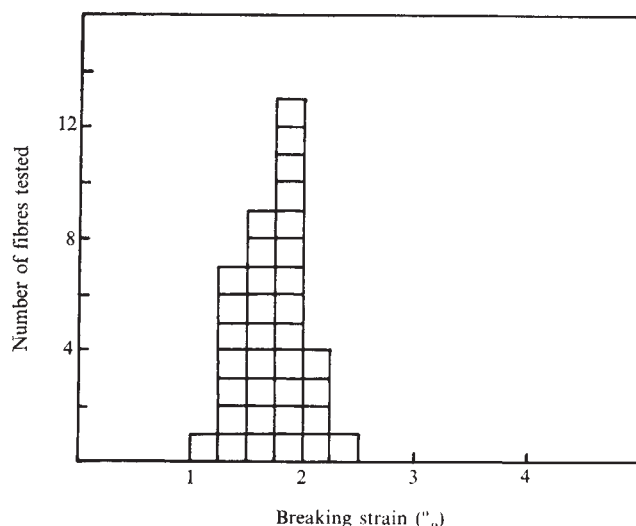


FIG. 4 Breaking strain distribution associated with fractures initiated at internal flaws.

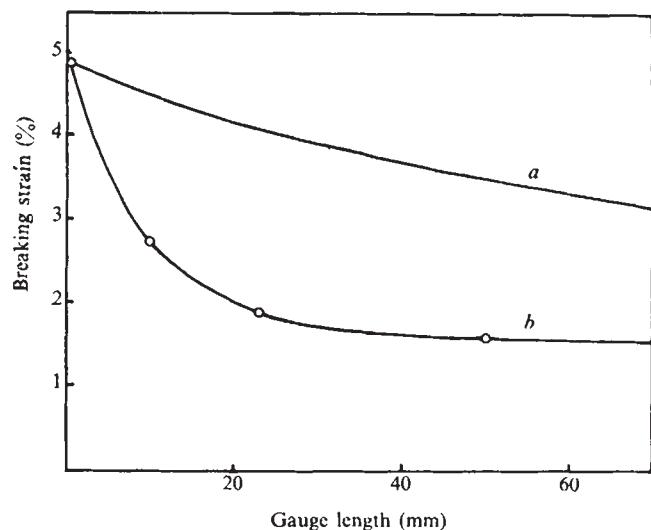


FIG. 5 Predicted (a) and experimental (b) mean breaking strain as functions of gauge length. Breaking strain standard errors are 0.13, 0.14, 0.06 and 0.07 for the four points on the curve in order of decreasing breaking strain.

are permitted to operate fully, then the gauge length effect can be predicted from their distribution in the original precursor acrylic¹ using

$$\epsilon = \epsilon_1 P + \epsilon_2 (1 - P)$$

where $\epsilon_1 = 4.9\%$ (loop tests), $\epsilon_2 = 1.71\%$ (Fig. 4) and P is the probability of avoiding an internal flaw for the particular gauge length^{1,13}. This is shown in Fig. 5, with the present experimental results. In spite of the improved breaking strains now achieved there is apparently a significant potential still to be tapped, even allowing for overestimation of loop breaking strain. The present shortfall in experimental values of breaking strain is partly explained by the persistence of some surface flaws causing fracture at low strain values, as indicated by the low strain tails of the distributions of Fig. 2. An example of such a fracture is illustrated in Fig. 1c. These particular surface flaws must be fairly well spaced at 50 to 100 mm and obviously contribute to the strong gauge length effect of Figs 2 and 5. In addition, they restrict the full operation of internal flaws, particularly at the longer gauge lengths. This is verified by the fracture frequency results of Table 2 where reasonable agreement between experimental and predicted frequencies is seen to extend only up to gauge lengths of 23 mm.

In addition to the achievement of breaking strains in excess of 2.0% at acceptable gauge lengths, the persistent operation of gross flaw mechanisms of fracture has been clearly demonstrated. There is no serious conflict between this treatment and the recent prediction of strength being dependent on ribbon wrinkling in graphitised fibre¹⁴. It has been recognised that structural features in addition to gross flaws may become progressively operative in controlling fracture in carbon fibre processed to graphitisation temperatures.

These studies confirm the earlier prediction on the feasibility of high breaking strain (2 to 3%) carbon fibres¹. They also form a satisfactory technical conclusion to the coherent and consistent treatment^{1,3,7,11-13} of carbon fibre strength and breaking strain on the basis of gross flaw mechanisms of brittle fracture applying at least to acrylic-based fibres processed to the carbonised stage. There remains scope for further development with the improvement of surface treatment techniques. The intrinsic breaking strain looks promisingly high, well in excess of 3.0%, and the strength correspondingly well above 6.0 GN m⁻² (0.9×10^6 pound inch⁻²).

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Crystal structure of polyvinyl alcohol

THE first comprehensive X-ray analysis of polyvinyl alcohol (PVOH) showed that the thirty observed reflections could be indexed according to a pseudo-orthorhombic cell¹. Bunn² later proposed a monoclinic unit cell comprising two molecules lattice parameters: $a = 7.81$ Å; $b = 2.52$ Å; $c = 5.51$ Å; $\beta = 91^\circ 42'$.

It was thought that the periodicity of 2.52 Å must imply an isotactic configuration with hydroxyl groups in identical positions along the backbone chain. This rather unlikely

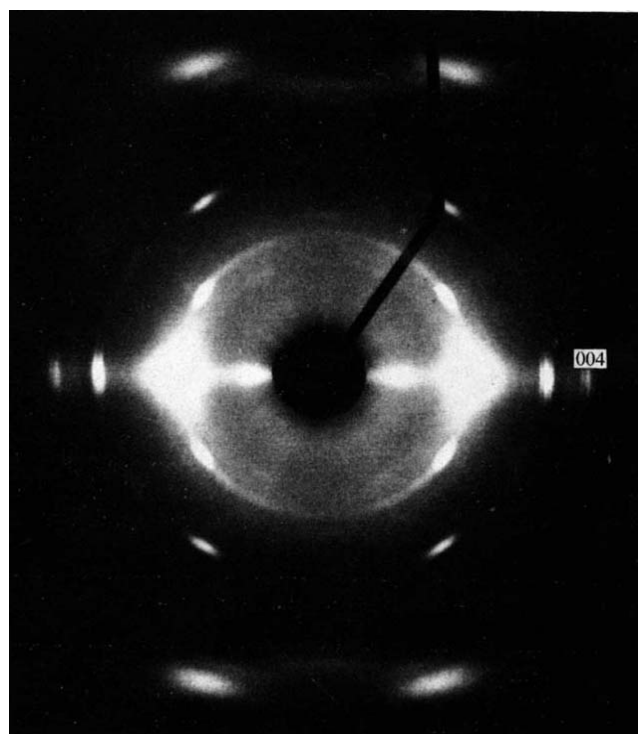


FIG. 1 X-ray diffraction photograph of crystalline PVOH fibre. 2θ for the 004 reflection is $33^\circ 40'$.

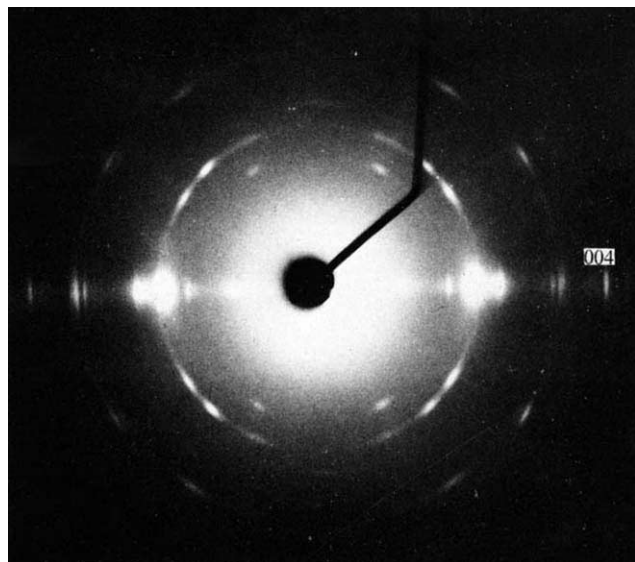


FIG. 2 X-ray diffraction photograph showing both crystal forms. 2θ for the 004 reflection is $33^\circ 40'$.

structure was modified by Bunn, who postulated that the repeat distance of 2.52 Å was "equally compatible with a molecular structure in which hydroxyl groups are randomly placed in left and right-handed positions".

Despite several later analyses³⁻⁵ the same basic lattice parameters and molecular configuration are widely accepted today. Mochizuki⁵ quoted a slightly higher fibre identity period of 2.533 Å which he obtained from a tilted-fibre photograph and, in agreement with Bunn, he considered PVOH to have a planar zigzag configuration. From an azimuthal scan of the so-called 100, 200 and 101 reflections, he found that as well as a maximum on the equator of the diffraction pattern, there was another at about 30° from the meridional axis. He therefore postulated that not all of the crystallites in an oriented fibre tend to arrange themselves with their long axis parallel to the fibre axis. Some are tilted by a very considerable angle (about 60°) to the direction of draw. No possible reason was given for this.

One of the most interesting problems encountered in the X-ray analysis of PVOH concerns the molecular conformation of stereoregular polymer. Both syndiotactic-rich and isotactic-rich PVOH have been prepared from the corresponding formate⁶. It is rather surprising, however, that no essential differences were noticed between their X-ray diffraction patterns—each showed an identity period of 2.5 Å.

I have found that the crystal structure of PVOH is somewhat more complex than has been previously realised. I used a commercial PVOH (Elvanol 71-30) to prepare highly drawn fibres because the chemical characteristics are advantageous for fibre production⁷. The more important qualities include a very high degree of hydrolysis (99%–99.8%), a suitable molecular weight (80,000) and a maximum ash content of 1%. A 20% aqueous solution of this polymer was extruded into a coagulation bath containing concentrated ammonium sulphate solution. No appreciable tension was applied at this

TABLE 2 Observed and calculated values of $\sin^2 \theta$ for the monoclinic cell

Index	Intensity	$\sin^2 \theta$ observed	$\sin^2 \theta$ calculated
200	m	0.009	0.009
002	m	0.021	0.021
202	vs	0.028	0.029
012/310	vs	0.032	0.031/0.032
400	m	0.039	0.039
020	m	0.041	0.041
203	s	0.060	0.059
022	vs	0.063	0.062
402	s	0.063	0.063
502	w	0.077	0.078
313	w	0.083	0.083
004	s	0.084	0.084
030	s	0.093	0.093
314	m	0.113	0.112
323	s	0.112	0.114
331	m	0.122	0.121
612	m	0.123	0.124
024	s	0.125	0.125
622/523	m	0.154	0.155
800	w	0.154	0.157
315	w	0.159	0.158
810	w	0.165	0.167
006	m	0.188	0.189
106	m	0.194	0.193
334	w	0.203	0.203
515/911	w	0.210	0.211
026/126	w	0.231	0.230/0.231
335/732	w	0.242	0.241
007	m	0.254	0.257
705	m	0.264	0.263
207	m	0.271	0.272
1200	w	0.351	0.353
060	m	0.372	0.372
906	m	0.406	0.406

w, weak; m, medium; s, strong; vs, very strong.

stage. After complete coagulation, a wet-drawing stage was incorporated in order to impart a degree of orientation sufficient to prevent dissolution of the fibres at the washing stage. After being collected and dried, the product was hot-drawn over a plate maintained at 200° C. Cylindrical and flat-film X-ray diffraction photographs were taken, using nickel-filtered $\text{CuK}\alpha$ radiation with exposure times from 1.5 to 4 h. Finely powdered copper was used as an internal standard, and the spacing of 2.088 Å was chosen as the reference line (American Society for Testing Materials, Powder Data File No. 4-0836).

X-ray diffraction photographs show many more reflections than have been previously noticed (Fig. 1). Of particular importance are those lying between the equator and the meridian, because they immediately indicate the existence of a somewhat larger unit cell than has been previously recognised. A detailed study of the diffraction pattern shows that no single unit cell can adequately account for all of the observed reflections. A system embodying two crystal structures will, however, account for them very satisfactorily. I therefore propose that polyvinyl alcohol crystallises in two forms which have the lattice parameters presented in Table 1. Tables 2 and 3 compare the observed and calculated values of $\sin^2 \theta$ based on these cells. The agreement is very good. Calculated density values agree with observed values for highly crystalline fibres. Assuming 18 monomers per orthorhombic unit cell, a density of 1.322 g cm^{-3} is obtained. For the monoclinic system, a density of 1.400 g cm^{-3} is obtained, assuming 24 monomers per cell.

Generally speaking, reflections belonging to the orthorhombic system are weak in intensity, presumably as a result of a structural preference for the monoclinic system. By altering both the total draw ratio and the temperature of the hotplate, fibre samples having varying proportions of the two crystal structures can be prepared. Figure 2, for ex-

TABLE 1 Lattice parameters of the two crystal structures of PVOH

α form (monoclinic)	β form (orthorhombic)
$a = 15.58 \text{ Å} \pm 0.02 \text{ Å}$	$a = 15.86 \text{ Å} \pm 0.03 \text{ Å}$
$b = 7.59 \text{ Å} \pm 0.01 \text{ Å}$	$b = 5.95 \text{ Å} \pm 0.02 \text{ Å}$
$c = 10.65 \text{ Å} \pm 0.02 \text{ Å}$	$c = 10.54 \text{ Å} \pm 0.03 \text{ Å}$
$\beta = 96^\circ 00' \pm 10'$	

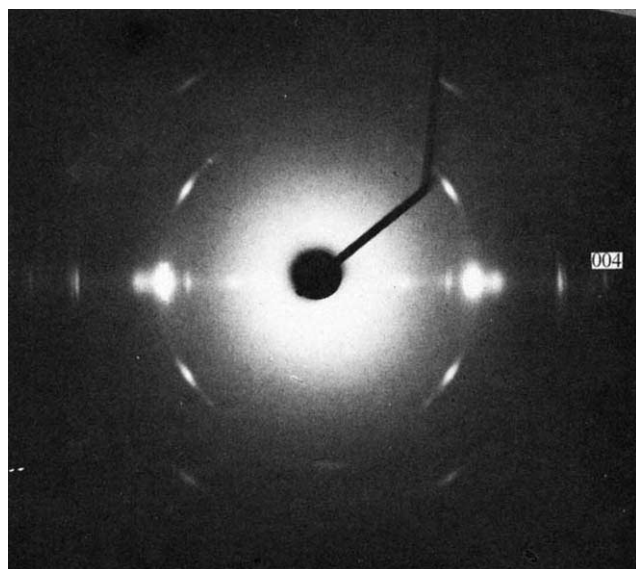


FIG. 3 X-ray diffraction photograph showing the monoclinic form only. 2θ for the 004 reflection is $33^\circ 40'$.

ample, shows reflections of up to 35° (2θ values) and exhibits both crystal forms. On the other hand, Fig. 3, consists only of reflections from the structure rich in the monoclinic form. Although the precise configuration of the polymer chains within the crystalline regions is unresolved at present, the fibre periodicities suggest a twisted or helical structure for both crystal forms. The monoclinic system, with a periodicity of $7.59 \text{ \AA}$, may be explained in two ways. It is possible that the polymer exists as a kinked chain in which every third hydroxyl group is in an equivalent position. Alternatively, if there is a high degree of syndiotacticity, a structure incorporating four monomer units per fibre repeat may be present. This latter type of configuration has been claimed for syndiotactic polypropylene⁸ and has been suggested for polyacry-

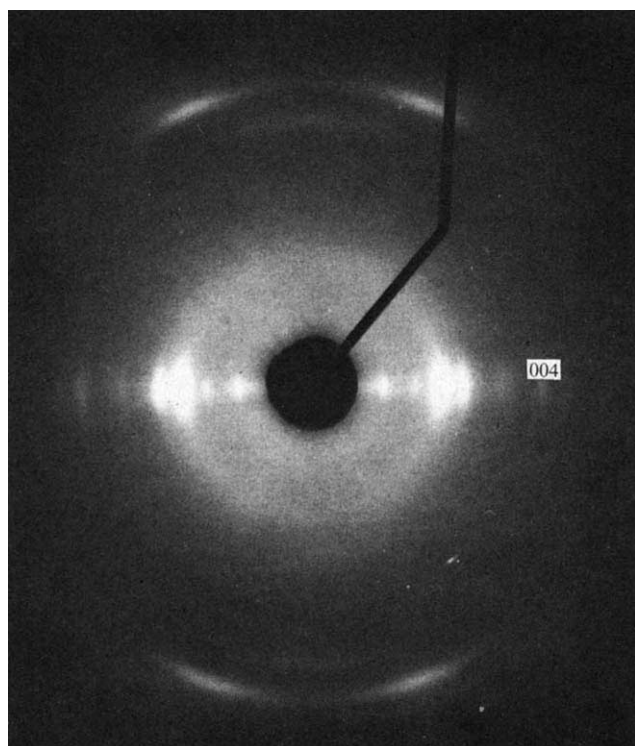


FIG. 4 Effect of water on the diffraction pattern of PVOH. 2θ for the 004 reflection is $33^\circ 40'$.

TABLE 3 Observed and calculated values of $\sin^2 \theta$ for the orthorhombic cell

Index	Intensity	$\sin^2$ observed	$\sin^2$ calculated
011	m	0.022	0.022
202	s	0.030	0.031
211	m	0.031	0.031
203	w	0.056	0.057
312	w	0.059	0.059
013	w	0.064	0.065
601	w	0.094	0.090
322	w	0.108	0.110
404	w	0.122	0.124
223	w	0.122	0.125
030	w	0.155	0.151
426	w	0.293	0.300

w, weak; m, medium; s, strong.

lonitrile (B. G. Colvin, and P. Storr, not yet published). These polymers have identity periods of $7.4 \text{ \AA}$ and $7.1 \text{ \AA}$ respectively. Furthermore, it is well known that the infra-red spectrum of PVOH shows a fairly high absorbance at 916 cm^{-1} , a wave number usually associated with the syndiotactic form. I feel, however, that the former proposal is in fact the more likely, because the 030 and 060 reflections are relatively strong and the 020 and 040 reflections are not visible at all (Table 1). It therefore seems that crystalline PVOH consists of a kinked chain with three monomers per repeat. The distortion of the main chain is regular and is apparently governed by a combination of mutual repulsion between neighbouring hydroxyl groups and a complex hydrogen-bonding mechanism.

It is reasonable to suppose that stereoregular PVOH may be examined more successfully provided that polymer samples of high crystalline order can be obtained. The following parameters are found to have a pronounced effect on the amount of detail manifested in a wide-angle X-ray diffraction pattern. First, the degree of jet-stretch is of some importance. This should be kept to a minimum, as it has been observed that a higher crystalline order is obtained by allowing the fibre to relax as much as possible in the coagulation bath. Second, a high degree of orientation is of the utmost importance, and this should be imparted principally at the wet-drawing stage. Third, dilute solutions of coagulant salts should be avoided as much as is practically possible. Water molecules adversely effect the naturally occurring hydrogen-bonding mechanism which is responsible for high degrees of order (Fig. 4), and washing stages should therefore be kept to a minimum. Even after crystallisation has occurred to a very high degree (Fig. 1), water molecules can penetrate the crystalline regions and thus modify the molecular packing arrangement. The only noticeable periodicity in the fibre direction after such treatment, results from the chemical repeat of $2.5 \text{ \AA}$. Presumably the kinked chain is no longer regular as a result of the disruption of hydrogen bonds within the polymer network. Crystallisation can still occur, however, but to a much lesser degree. The small size of the hydroxyl group is probably the reason that PVOH can crystallise at all under these conditions². Whether there is any marked variation in the lateral packing of chains is uncertain at present, although no pronounced variations in the equatorial reflections have been noticed. It is proposed that more information may become available from fibres of varying tacticity produced in the near absence of water. The use of organic precipitating baths may prove useful in this respect.

This analysis also suggests that there is now no real evidence to support a 'preferred tilted-crystal orientation' theory, because an azimuthal scan of the so-called 100, 200 and 101 reflections would necessarily give rise to other maxima. These, however, arise from quite separate reflections, namely the 012 or 310 of the monoclinic system and the 211 of the

orthorhombic cell. It must be presumed that Mochizuki⁵ had prepared a sample of PVOH showing some signs of increased crystalline order but, instead of assigning separate indices to the off-equatorial maxima, he included them as part of the equatorial arcs.

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Estimation of solubility parameters of nonelectrolytes

THE Hildebrand approach^{1,2} provides an explanation of the solubility of nonelectrolytes based on a thermodynamic method. In this method used for the estimation of solubility, it is assumed that each molecule possesses a characteristic potential energy based on intermolecular forces which may be described as the cohesive energy density. The latter may be defined as the potential energy of 1 cm³ of liquid or the energy required to separate all molecules in a unit volume of liquid. This value is zero when the molecules are randomly dispersed in a solution.

The solution process may be compared with the vaporisation process in which molecules at an equilibrium distance are transported to an infinite distance. Accordingly, the energy of vaporisation is used to quantify the solution process in the Hildebrand approach. Thus, the solubility parameter which is represented by the symbol δ is equal to the square root of the cohesive energy density (CED). The latter is equal to $\Delta E_v/V$ in which ΔE_v is the energy of vaporisation and V is the molar volume.

As shown by the following equations, values for the solubility parameter (δ) which is the square root of CED, may be calculated from the heat of vaporisation per mole (ΔH_v), the gas constant (R), the temperature on the Kelvin scale (T) and the molar volume (V):

$$\begin{aligned}\delta &= (\text{CED})^{1/2} = \left(\frac{\Delta E_v}{V}\right)^{1/2} = \left(\frac{\Delta H_v - RT}{V}\right)^{1/2} \\ &= \left(\frac{\Delta H_v - RT}{M/D}\right)^{1/2} = \left(\frac{D(\Delta H_v - RT)}{M}\right)^{1/2}\end{aligned}$$

The above equation is derived from the following relationships:

$$\Delta H_v = \Delta E_v + P\Delta V = \Delta E_v + RT \therefore \Delta E_v = \Delta H_v - RT$$

$$D = M/V \therefore V = M/D$$

Because of the difficulty in obtaining values for the molar volume, the quotient of the gram formula weight (M) and density (D) is substituted for the value V . The units for δ values obtained from the preceding equation are (calorie cm⁻³)^{1/2} but the term hildebrand has been adopted in some instances for this unit³. According to the solubility parameter concept, solubility occurs when the δ values of solid solute and a liquid solvent are within a range of 2 hildebrands (2 H).

The quality of hydrocarbon solvents such as benzene, cyclohexane or hexane is readily described by δ values and these nonpolar solvents are called regular solvents. But the potential energy of solvents increases with polarity because of additional interactions resulting from dipole-dipole (Keesom) forces, induced dipole-dipole (Debye) forces and acid-base type (Lewis) forces and provisions must be made to account for these additional interactions in applications of the solubility parameter concept. Burrell⁴ has classified solvents as poor, moderately bonded and strongly bonded solvents.

Several graphical approaches have been suggested for the estimation of δ values. Values of ΔH at 298 K may be estimated from the boiling points (T_b) and these values may be used for the calculation of δ values by use of the following equation⁴:

$$\Delta H_{298} = 23.7T_b + 0.02T_b^2 - 2,950$$

(see Fig. 1).

It has also been shown previously that the contribution of the methylene group (CH₂) to the δ values is constant⁵ and so the δ values for solvents in a homologous series may be estimated from the graph shown in Fig. 2 (ref. 6).

The logarithm of δ values of nonpolar solvents such as hexane ($\delta = 7.3$), cyclohexane ($\delta = 8.2$) and benzene ($\delta = 9.2$) are related to readily obtainable physical constants as shown by the following equation and by line A in Fig. 3;

$$\log \delta \simeq 0.50 \log \frac{T_b D}{M} + 0.655$$

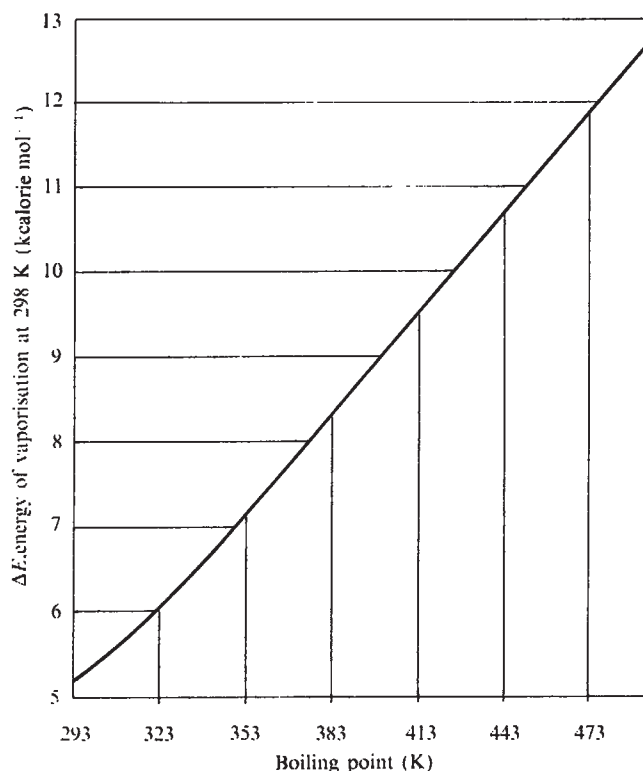


FIG. 1 Relationship of ΔE and boiling point.

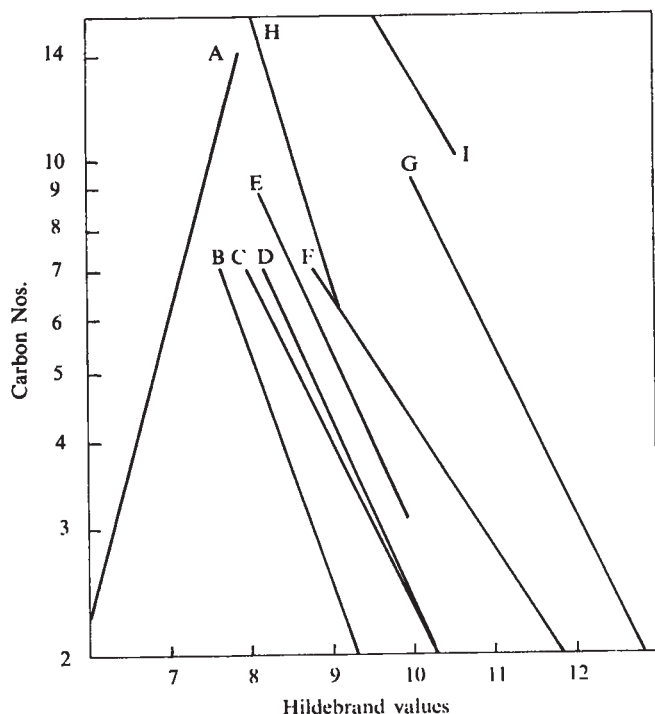


FIG. 2 Relationship of solubility parameters and carbon numbers in various homologous series of solvents. A, Normal alkanes; B, normal chloroalkanes; C, methyl esters; D, alkyl formates and acetates; E, methyl ketones; F, alkyl nitriles; G, normal alkanols; H, alkyl benzenes; I, dialkyl phthalates.

A slightly different equation may be used to estimate the logarithm of δ values for moderately bonded solvents as shown below:

$$\log \delta \cong 0.40 \log \frac{T_b D}{M} + 0.665$$

These values which apply for solvents such as ethyl ether ($\delta = 7.4$), butyl acetate ($\delta = 8.0$), acetone ($\delta = 9.9$) and dimethyl sulphoxide ($\delta = 12.0$) are shown as line B in Fig. 3.

The logarithm of δ values of strongly hydrogen bonded solvents may be estimated from line C in Fig. 3 or from:

$$\log \delta \cong 0.40 \log \frac{T_b D}{M}$$

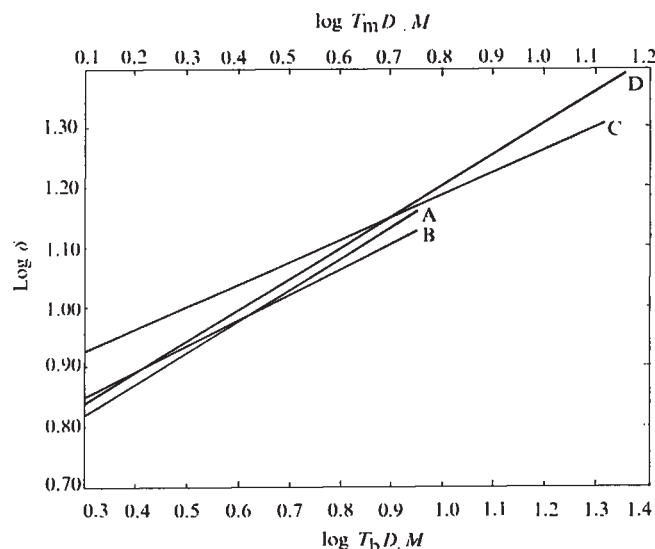


FIG. 3 Relationship of $\log \delta$ and $\log$ of readily available constants.

Typical strongly hydrogen bonded solvents are diethylamine ($\delta = 8.0$), sec. amyl alcohol ($\delta = 10.0$), acetic acid ($\delta = 10.1$), and aniline ($\delta = 10.3$). Because they exist primarily as dimers, the M values used for formic and acetic acids are twice the molecular weights of the simple molecules.

The logarithm of δ values of solids such as naphthalene ($\delta = 9.9$), β -bromonaphthalene ($\delta = 10.6$) and maleic anhydride ($\delta = 13.6$) may be estimated from line D in Fig. 3 and from:

$$\log \delta \cong 0.50 \log \frac{T_m D}{M} + 0.670$$

Tables of solubility parameter values are readily available⁴. As shown by the following example, solubility parameters may also be readily calculated from the graphs included in this report. The boiling point of carbon tetrachloride is 77°C or 350 K. According to Fig. 1, the value of ΔE for carbon tetrachloride is 7.2 kcal/mol. Therefore, by use of the relationship

$$\delta = \left(\frac{\Delta E}{V} \right)^{1/2} = \left(\frac{D(\Delta E)}{M} \right)^{1/2} = \left(\frac{7,200(1.6)}{154} \right)^{1/2}$$

one obtains a δ value of about 8.4 H for carbon tetrachloride.

Similarly, according to line H in Fig. 2, the δ value for toluene ($C = 7$) would be about 8.9. The scale for the ordinate is an arbitrary one chosen so that linear relationships could be demonstrated when the number of carbon atoms in the solvent was plotted against the solubility parameter values in hildebrand units. The δ value of a strongly hydrogen bonded solvent such as methanol may be estimated from line C in Fig. 3 by using the values $T_b = 338$ K, $D = 0.79$ g/cm³, $M = 32$. So

$$\log \frac{T_b D}{M} = 0.922$$

Thus, $\log \delta = 1.16$ and $\delta = 14.5$ H.

In addition to providing quantitative values in place of the qualitative expression, 'like dissolves like,' the solubility parameter may be used to explain that while cellulose nitrate ($\delta = 10.5$ H) is insoluble in either ethanol ($\delta = 12.7$ H) or ethyl ether ($\delta = 7.94$), it is readily soluble in an equimolar mixture of these two solvents ($\delta = 10.0$ H). This solution, called collodion or newskin, is still in use today.

The solubility parameter may also be used to explain many well known unique solvent systems such as a saturated aqueous solution of urea. This solution of natural origin has been used to dissolve dried blood, to soften animal skins, to set hair, to disperse the ingredients of gun powder, to aid the inking of lithographic plates, to make wood flexible and to improve the flow of cold house paints.

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Applications of Boolean programming optimisation methodology to the direct solution of crystal structures

We have found that Boolean optimisation methods can be used to derive phase information from observed X-ray structure factors. This will provide a new approach to the problem of the loss of phase information during X-ray diffraction experiments, which has been a major limitation to the study of the structure of matter. The possibilities of the method are demonstrated here in the successful application of integer programming optimisation techniques to the three-dimensional solution of centrosymmetric structures.

Current direct methods¹⁻⁵ involve statistical relationships among a group of reflexions preselected to be strongly influenced by the atomic arrangement. The phase problem assumes two levels of complexity depending on (1) the absence or (2) the presence of a centre of symmetry. (1) In the more complex general case, the phase angle is free to assume all values from 0° to 360°. (2) The presence of a crystallographic centre of symmetry constrains the phase angle to 0° or 180°, and the cosine of the phase angle is therefore constrained to ± 1 .

The phase relationships can be formulated in terms of classical integer programming. For the centrosymmetric case, the variable, X , equal to zero or one, corresponds to a sign or $-$ or $+$ (for example, sign = $-(-1)^X$). Freeman⁶ and Dakin⁷ have explored the use of integer programming techniques in the one-dimensional case, and Lesk⁸ redetermined the planar structure of dihydrouacil in a two-dimensional integer programming formulation. We now report the application of integer programming techniques in three dimensions to the solution of the hitherto unstudied molecule, spiro-[5.5]-undeca-1,4,7,10-tetraene-3,9-dione⁹, 'spirodienone'. We have also redetermined the structure of potassium lead hexanitrate(II), $K_2PbCu(NO_3)_6$, using the structure factors of Cullen and Lingafelter¹⁰.

We have found that certain integer programming methods fail when identical constraints are used. Therefore, a disjoint set of inequality constraints was chosen to place upper and lower bounds on the values of electron density at points in the crystallographic unit cell. For the spirodienone study, choice of these points was assisted by a procedure similar to the application of the symmetry minimum function to Patterson(vector) space¹¹. Pronounced valleys are located on the map of the Patterson function and a valley in vector space is related to positions in real space where no atom is expected to appear. For the centrosymmetric case, a point of minimum density in vector space at $2x, 2y, 2z$ can be mapped into real space at x, y, z , together with the related points within the unit cell generated by symmetry and translations. For space group $P\bar{1}$, points need to be mapped into only half the unit cell, which is the asymmetric unit. In higher symmetry space groups, the location of local minima in Harker lines and sections would help establish positions in real space for the assignment of constraints.

A typical constraint in space group $P\bar{1}$ can be constructed by setting bounds to the inequalities:

$$\sum_{j=1}^n A_{ij}X_j - b_i \leq \text{upper bound and}$$

$$\sum_{j=1}^n A_{ij}X_j - b_i \geq \text{lower bound.}$$

The index, i , refers to a point in real space; j refers to a reflexion. The variable X_j assumes binary values zero or one as the phase of reflexion j is assigned a value of 180° or 0°. The coefficient A_{ij} represents twice the value of the computed electron density at point i from structure factor j , (assuming a positive sign for each F value) that is $A_{ij} = (4/V) |F_j| \cos 2\pi(h_jx_i + k_jy_i + l_jz_i)$, where V is the volume of the unit cell. The term b_i is evaluated to be half the sum of all the terms A_{ij} at point i for n reflexions: that

is, $b_i = 1/2 \sum_{j=1}^n A_{ij}$. The upper and lower bounds are

near-zero-valued parameters which define a window within the computed electron density summation must fit. The assignment of a value of one to phasing term X_j has the net effect of adjusting the electron density by the contribution of the positive structure factor j at that point. Conversely, if X_j is equal to zero, the effect is to adjust the contribution of reflexion j to the electron density due to the negative sign.

If the choice of variables X_j corresponded with the correct set of signs, the left-hand side of the inequality constraints would approximate the actual electron density at each point where the constraint is computed. Deviations from the actual electron density may be attributed both to errors inherent in the data set (which would usually be minimal) as well as to the incomplete nature of the data set due to limitations in computer size. When the objective function and a set of constraints are input to the surrogate constraint Zero-One Algorithm¹² then all feasible sets of phases which are found on the way to the optimum solution are output. For spirodienone, the coefficients in the objective function were biased by the computed value of the electron density due to each reflexion in the Fourier summation at the starting position. Then optimisation techniques 'tour' solution space in search of the optimum phases.

For the first trial of the method we chose spirodienone because of its structural interests to spectroscopists and its suitability for our procedure. Crystals of spirodienone, $C_{11}H_{10}O_2$, were grown from a dioxane solution of pure compound provided by Dr Guy Farges. Cell parameters are:

$$\begin{array}{lll} a = 9.08 \text{ \AA} & b = 13.61 \text{ \AA} & c = 7.49 \text{ \AA} \\ \alpha = 91.11^\circ & \beta = 96.91^\circ & \gamma = 100.50^\circ \end{array}$$

$P\bar{1}$, $Z = 4$. Thus there are two independent molecules of 13 non-hydrogen atoms in the asymmetric unit. The diffractometer data were reduced to normalised structure factors (E values). An analysis of the E values showed the presence of a strong subcell, therefore the structure was first solved by a direct methods program, MULTAN⁵, to establish that a solution would be forthcoming.

The method worked well when it was provided with the starting position of a single atom (taken from a peak of the MULTAN E map) to define the origin of the unit cell. The coefficients in the objective function were biased by the computed value of electron density due to each reflexion in the Fourier summation at that known position. For 170 variables (signs) and 160 constraints a near optimum solution was found within 80s on a CDC 6600 computer. The resulting 170 signed E values were input to a Fourier program and the resulting E map clearly indicated twenty-three of the twenty-six non-hydrogen peaks. Four cycles of full matrix least squares refinement of the twenty-three peaks produced an R factor of 30.0% (based on F) and a new set of signs that, in the next Fourier calculation, yielded the remaining three atoms. All non-hydrogen atoms together with their isotropic temperature factors refined to an R factor of 13.9%. Further refinement has lowered R to 4.8%.

We were not satisfied that *a priori* information would be a necessary condition for the proper functioning of the

optimisation approach. We therefore redetermined a known structure, this time in the cubic crystal class, with several heavy atoms (Pb, K, Cu) as well as light atoms (N, O) in the molecule. Potassium lead hexanitrocuprate (II) crystallises in space group $Fm\bar{3}$, $a_0 = 10.672\text{\AA}$.

Normalised structure factors were generated from the observed reflexions. The statistical distribution of the E values was highly uncharacteristic for both centric and acentric cases. The highest E was only 1.85. The application of the optimisation approach produced the correct structure in a straightforward manner.

The fifteen atomic parameters were sought by applying ninety lower-bound constraints on electron density. All points were chosen from an $0.5\text{\AA}$ grid in the asymmetric unit without reference to the Patterson map, as in the first structure. Fifty structure factors corresponding to the highest thirty-five E values in the parity group *ggg* and the highest fifteen in the other parity group, *uuu*, were used. The signs thus determined were used in a Fourier summation which yielded the correct structure. This second solution thus demonstrates that Boolean optimisation methods can derive phase information directly from the observed structure factors without *a priori* information. As these two analyses are our first two attempts to test the strengths and limitations of this approach, the results are encouraging. However, more study is required to define the critical parameters of the constraint's expression and the limitations of the method itself.

In conclusion, two analyses of the phase problem in the centrosymmetric case have been made. In the first trial, one atom at a known position was required to bring about phasing convergence. Although as mentioned, *a priori* information was required, this trial suggests the possibilities of this method for phase refinement or phase extension. As a second trial we chose a molecule in the cubic class. The method was able to proceed directly from structure factors to signs without *a priori* assumptions or information. Thus, a nonstatistical approach to direct methods is possible by means of Boolean programming optimisation methods.

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BIOLOGICAL SCIENCES

Premature ageing and occurrence of altered enzyme in Werner's syndrome fibroblasts

HUMAN diploid fibroblasts cannot be propagated indefinitely in culture¹. Longevity experiments with fibroblasts grown from skin biopsies from individuals of different age show that the greater the age of the donor, the shorter the lifespan of their cells in culture²⁻⁴. Patients suffering from Werner's syndrome age prematurely and have an average lifespan of 46 yr (ref. 5). Martin, Epstein and Sprague⁴ established primary fibroblast cultures from skin specimens from four such patients and discovered that these could be subcultured only 4-11 times before growth ceased. This compares with an average lifespan of 32 subcultures for skin fibroblasts from normal individuals in the same decades of life⁴. We show here that changes which occur in the enzyme glucose-6-phosphate dehydrogenase (G6PD) during the senescence of normal fibroblasts⁶, also occur during the premature senescence of cells from a patient with Werner's syndrome.

Werner's syndrome is an inherited condition, caused by a rare autosomal recessive allele⁵. A review and critical appraisal of the similarities between normal ageing and premature ageing in these patients is available⁵. The features of Werner's syndrome are first seen soon after adolescence. Normal development is then interrupted by greying of the hair and sometimes baldness, impairment of further bodily growth, loss of subcutaneous tissue and muscle, and progressive changes in the skin, all of which give the affected individual a prematurely aged appearance. Other changes which typically take place include bilateral cataracts, weakness of the voice and underdevelopment of the genitalia with consequent low level of fertility. Arteriosclerosis becomes manifest and calcium is deposited in arterial walls. The development of mild diabetes is common, and there is also an unusually high incidence of neoplasms. The combination

TABLE 1 Estimates of the heat-labile fraction of G6PD in Werner's syndrome cells, controls and MRC-5 cultures of different age.

Culture	Donor	Passage No.	% Heat-labile enzyme
Werner's skin fibroblasts	Male, age 46	7	21
		9	23
		9	22
		11	22
		11	21
Normal skin	Male, age 54	11	9
		25	8
		27	6
	Female, age 40	8	4
		22	4
Normal MRC-5 foetal lung fibroblasts	Male	10-40 (27 experiments)	range: 0-10 mean: 5.8
Senescent MRC-5	Male	53-68 (34 experiments)	range: 10-27 mean: 16.7

Each determination is based on an experiment of the type shown in Fig. 1. The summary of results with MRC-5 are taken from Holliday and Tarrant⁶.

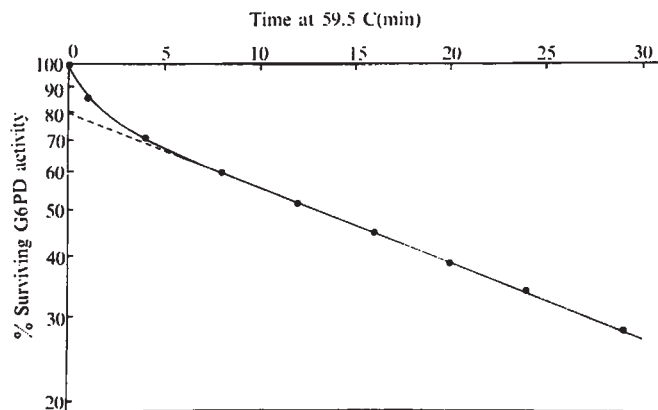


FIG. 1 The inactivation by heat of G6PD in a cell-free extract from passage 11 skin fibroblasts, derived from a patient with Werner's syndrome. The methods for the preparation of extracts and the assay of the enzyme are as previously described⁶.

of subcutaneous wasting with taut and shiny skin results in spindly extremities, which contrast with a stocky trunk. Callosities and later ulcers develop on the ankles as well as over the Achilles tendons of the heels. Although these ulcers are particularly indolent and resistant to effective treatment, vascular insufficiency does not seem to be responsible. Their failure to heal probably reflects the limited division potential of cells which normally replace tissue over these areas which are vulnerable to pressure and damage.

Several of these features in a male patient (DD), aged 40, led to the diagnosis of Werner's syndrome in 1966 (ref. 7). In July 1972 specimens of skin and subcutaneous tissue were taken from the forearm and samples were used to establish primary fibroblast cultures. Small pieces of tissue were placed under coverslips in Leighton tubes and incubated at 37° C in Eagle's basal medium containing 10% foetal calf serum and 10% tryptose phosphate broth. Half the medium was changed twice weekly. When sufficient cells had migrated from the tissue and grown to form a monolayer, they were removed after trypsin treatment and subcultured into small glass bottles. In some cases several populations of cells could be collected at intervals of 2–3 weeks from the same Leighton tube. The cells were passaged with a 1:2 split ratio using the standard procedures for fibroblast cultures⁸, although growth of the Werner's cells was considerably slower than with normal fibroblasts. Of the various cultures established, none survived beyond passage 12. This is in agreement with the results obtained by Martin *et al.*⁴.

Sufficient cells were obtained to carry out several experiments with G6PD, but there was insufficient material to examine any other enzyme. It has previously been shown that healthy cultures of foetal lung strain MRC-5 contain on average about 5% of altered G6PD which is heat labile, whereas senescent cultures can obtain up to 27%, with an average of about 17%⁶. Although it is not ruled out that this altered enzyme is produced by post-synthetic modification, the overall evidence indicates that the errors in protein synthesis are responsible for the changes in the enzyme which are seen during ageing⁶. Five experiments were carried out with cell-free extracts from cultures of Werner's cells, and in each case about 20% heat-labile enzyme was detected. One of these experiments is shown in Fig. 1, and the overall results are given in Table 1. The estimate of the proportion of altered G6PD is accurate to within about 3%. We have examined G6PD from cultured fibroblasts from three normal adults. In each case the amount of altered G6PD is in the same range as non-senescent MRC-5 cultures. These results are given in Table 1. A mixture of a cell-free extract from Werner's cells (21% heat-labile enzyme) and an equal amount of control enzyme (9% heat-labile) gave

the expected intermediate level of altered G6PD (16% heat-labile).

Our results with G6PD show that cells from Werner's patients have at least one biochemical defect in common with senescent fibroblasts derived from a normal individual, and this strengthens the view that the early death of Werner's cells in culture is really due to premature ageing, and makes it more likely that there is a direct relationship between *in vivo* and *in vitro* ageing. The results also add weight to the growing evidence that ageing is associated with the production of defective proteins^{6,8–14}, although so far there is little to indicate whether this is a primary cause of ageing or a secondary consequence¹⁵. One possibility is that the altered proteins arise from an ever increasing level of errors in protein synthesis, as originally suggested by Orgel¹⁶. A genetic defect which accelerated this process could be due to a loss of specificity of one of the many enzymes, or ribosomal proteins, which maintain the normal accuracy of protein synthesis. Most mutations of this type, however, would be dominant or partially so. Recessive mutations are frequently associated with the loss of an enzyme activity; therefore, if the recessive genetic defect causing Werner's syndrome is due to a slight change in the accuracy of protein synthesis, one possibility is that an enzyme which modifies bases in tRNA or rRNA is inactive or absent.

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Life span of erythrocyte membrane protein

IN most types of cells the protein constituents of the cytoplasm are constantly being degraded and synthesised^{1–6}. When the proteins in these cells are labelled with a protein precursor, the label disappears from the protein with first order kinetics. The amount of any protein in the cell then is regulated by the rate of its synthesis and degradation, and recent work indicates the importance of this process as a regulatory mechanism in mammalian cells. In contrast, when haemoglobin, which comprises 90% of the protein of the mature mammalian erythrocyte, is labelled, it does not decay with first order kinetics, but survives for a finite period. It

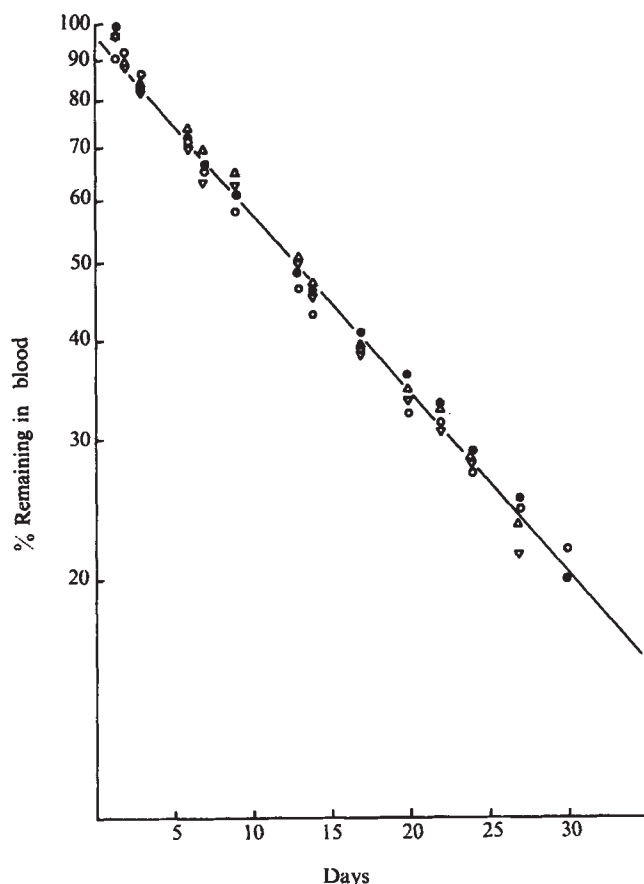


FIG. 1 Survival curves of ^{51}Cr and ^{125}I -labelled rat erythrocytes *in vivo*. Labelled cells (1 ml) were injected through the tail vein. At various times, blood samples were taken by tail cut and 0.05 ml of blood was collected into a heparinised capillary tube and mixed with 1.0 ml of normal saline. Samples were counted on a Packard gamma spectrometer. Four combinations of isotope were used: $\circ$, ^{125}I ; $\bullet$, ^{125}I Cr; $\triangle$, ^{51}Cr I; and ∇ , ^{51}Cr . Each point is an average of data for four rats corrected for the decay of the isotope used.

disappears from the vascular bed when the intact erythrocyte is removed from circulation. Thus, haemoglobin labels provide excellent material for studies of the life span of the red cell.⁷

Membrane proteins also turn over in most animal cells. In the liver, for example, the half life of the plasma membrane proteins is very short¹⁻⁶. Efforts to label the membrane proteins of the erythrocyte and determine whether it survives similarly for the lifetime of the cell have not been completely successful⁸. Using ^{14}C -glucose which is incorporated into glycopospholipids, Krivit⁹, however, found a mean life span for rabbit red cells of 51 to 60d. These values are similar to but shorter than those obtained by labelling haemoglobin in the rabbit¹⁰. We have now labelled rat erythrocytes by a method previously described¹¹⁻¹³ and have studied the survival of their membrane proteins *in vivo*.

Male Sprague Dawley rats (200 ± 10 g) were fed a regular diet, given water *ad lib* and housed four or five to a cage. Blood was drawn into plastic syringes containing ACD anticoagulant (0.15 ml ml^{-1} of blood) from rats anaesthetised with ether. The blood was then placed in plastic tubes. The red cells were separated by centrifugation at $750g$ for 10 min and the serum was removed. For labelling with chromium^{14,15}, packed cells were obtained from 10 ml of blood. Potassium chromate (10λ , 1 mg ml^{-1}) was added with stirring. After incubation at 37°C for 40 min, 0.1 ml of 0.1 M sodium ascorbate solution was added. The cells were incubated for a further 10 min and washed three times in isotonic saline.

To label cells with ^{125}I , packed cells were washed free of

plasma protein in isotonic phosphate buffer. The washed cells were then subjected to lactoperoxidase-catalysed iodination as previously described¹¹⁻¹³, centrifuged, washed three times in isotonic saline, suspended in serum and left overnight. Cells were then centrifuged free of the serum, washed twice in isotonic saline and suspended in saline to give a 50% haematocrit. The cells were also double labelled with either ^{125}I and non-radioactive chromium or ^{51}Cr and non-radioactive iodine. In all cases chromium labelling preceded labelling with iodine.

The cells from four groups of rats, consisting of four animals each were labelled with ^{125}I , ^{51}Cr , ^{125}I and non-radioactive chromium, or ^{51}Cr and non-radioactive iodine, as described above. A sample of 1 ml of cells was injected into the tail vein of each animal, and blood samples were obtained at various times thereafter. The amount of label present in the blood of each of the four groups was followed for 1 month.

As much as 25% of the total label rapidly disappeared within the first few hours after injection of cells. However, the rate of decay became more linear after 20h. Figure 1 shows the decay curve for the disappearance of radioactive label, corrected for the half-life of the isotopes used, from the four groups of animals used. The similarity in the rate of decay between ^{51}Cr and ^{125}I suggests that the label in exposed membrane proteins and that of internal components were disappearing in parallel.

Because ^{51}Cr elutes from the red cell it does not provide a simple relationship to the mean red cell life span^{14,15}. Survival times determined by chromium underestimate the mean red cell life span unless corrected. Since the disappearance of membrane label coincided with chromium decay, the data suggested that iodine was also being lost from the cell. To investigate whether any one of the membrane components is involved in a selective loss of an iodine-labelled component, erythrocyte membranes were isolated and polypeptides were separated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis.

Previous studies has shown that two proteins on the exterior surface of the human erythrocyte are labelled with the lactoperoxidase system¹¹⁻¹³. One has a molecular weight of 90,000 and the other is the major glycoprotein. In the rat, five polypeptides are iodinated. The major component labelled is in the 90,000 dalton class. The minor components have molecular weights of 68,000, 31,000, 18,000 and include an additional component with a low molecular weight, probably 12,000 or smaller.

As Fig. 2 shows, although there was a marked loss of radioactivity, there does not seem to have been a selective loss of any of the components. The minor components disappeared or were not readily detected after 404 h, because of the small amount of iodine originally incorporated.

The data suggest that labelling of the erythrocyte with the lactoperoxidase probe provides a method for the study of red cell survival, but does not offer any distinct advantages over present procedures. The labelling procedure is somewhat more complicated than the use of chromium. The iodine label gives results which indicate that a significant portion of the iodine label is removed from the cell during its life in the vascular system. The reasons for this are not clear. Chromium and other labelling systems label primarily the haemoglobin molecule which does not turn over as do proteins in other cell types, but survives for the life span of the red cell and is lost primarily by removal of the intact cell.

In other types of cells there is a marked difference in the rate of turn over of individual proteins of a membrane system¹⁻⁶. Analysis of the membrane components of the rat erythrocyte does not suggest any great difference in the rate of disappearance of the labelled membrane polypeptides. In other types of cell there seems to be a correlation between the size of a protein and its rate of degradation *in vivo*. The

(Continued on page 789)

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxy-ribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's² model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

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The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{3,4} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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April 2.

The double helix: a personal view

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Francis Crick reviews the papers published 21 years ago on the structure of DNA and the reaction to them.

For this anniversary I thought it might be appropriate to look back, in a rather informal way, at the original papers on the structure of DNA to see how they appear today in the light of 21 years of research.

During the spring and summer of 1953 Jim Watson and I wrote four papers on the structure and function of DNA. The first appeared in *Nature* on April 25 accompanied by two papers from King's College London, the first by Wilkins, Stokes and Wilson, the other by Franklin and Gosling. Five weeks later we published a second paper in *Nature*, this time on the genetic implications of the structure. A general discussion was included in the volume that came from that year's Cold Spring Harbor Symposium, the subject of which was viruses. We also published a detailed technical account of the structure, with rough coordinates, in an obscure journal¹ in the middle of 1954.

The first *Nature* paper was both brief and restrained. Apart from the structure itself the only feature of the paper which has excited comment was the short sentence: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material". This has been described as 'coy', a word that few would normally associate with either of the authors, at least in their scientific work. In fact it was a compromise, reflecting a difference of opinion. I was keen that the paper should discuss the genetic implications. Watson was against it. He suffered from periodic fears that the structure might be wrong and that he had made an ass of himself. I yielded to his point of view but insisted that something be put in the paper, otherwise someone else would certainly write to make the suggestion, assuming we had been too blind to see it. In short, it was a claim to priority.

Why, then, did we change our minds and, within only a few weeks, write the more speculative paper of May 30? The main reason was that when we sent the first draft of our initial paper to King's College we had not yet seen their own papers. Consequently we had little idea of how strongly their X-ray evidence supported our structure. The famous 'helical' X-ray picture of the B form, reproduced by Franklin and Gosling in their paper, had been shown to Watson, but he certainly had not remembered enough details to construct the arguments about Bessel functions and distances which the experimentalist gave. I myself, at that time, had not seen the picture at all. Consequently we were mildly surprised to discover that they had got so far and delighted to see how well their evidence supported our idea. Thus emboldened, Watson was easily persuaded that we should write a second paper.

The papers in *Nature*

The two experimental papers of April 25 overlap to a considerable extent. Rosalind Franklin's paper mentions the

crystalline A structure, but only briefly, except for the claim that the Patterson superposition function (which was in the press at the time) supported two chains rather than three. Both papers stress that there must be more than one chain in the structure. Indeed Maurice Wilkins had personally told Chargaff that a year or so earlier. Both present the argument that the positions of the intensity maxima ruled out two (parallel) chains related by a dyad parallel to the fibre axis. Neither gave the neat argument, due to Watson, that their own density measurement, together with the observed change in length between the two forms, supported two chains rather than three. Franklin noted that if there were several chains they could not be equally spaced and that 'equivalence' favoured two rather than three. It was not explicitly stated, however, that equivalence implies dyad axes perpendicular to the fibre axis and that therefore the two chains must run in opposite directions. Nor did she realise that the monoclinic unit cell of the A form also suggested this, although we had deduced this from her own experimental data.

Both papers correctly concluded from the intensity positions that the phosphate-sugar backbone was on the outside of the structure and that the bases were stacked on the inside. Franklin repeated the argument, which she had made to us verbally a year earlier, that the phosphates would be hydrated (in which she was perfectly right) and therefore that they would probably be on the outside of the molecule. In short, both the groups at King's College had obtained a fairly general idea of the structure but they had done no proper model building. Mainly because of this they had missed the pairing of the bases and they had completely overlooked the significance of Chargaff's rule.

The omissions in the paper by Watson and myself are also striking. The structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it. No dimensions are given (let alone coordinates) except that the base pairs were 3.4 Å apart and that the structure had 10 base pairs in its repeat. The exact nature of the base pairing was not immediately obvious; nor even unambiguous since at that time there were two systems for numbering pyrimidine rings. Most of this information was provided in the subsequent papers. However the general nature of the structure was clear enough, though the tone of the paper ("it must be regarded as unproved until it has been checked against more exact results") was, apart from the short first paragraph, rather muted.

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MOLECULAR BIOLOGY AND METAPHYSICS (G. S. Stent) . . .	779
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Although a casual reader could easily have overlooked the significance of the first set of papers, especially as they were full of obscure crystallographic jargon, he could hardly miss the impact of our second one. The biologically important features of the proposed structure were explicitly described. The base pairs were listed with the minimum of hedging about tautomerism and were illustrated in scale diagrams. The proposed duplication mechanism was spelt out in simple terms, unmarred by any trace of algebra. In spite of the discussion of the difficulties of unwinding, the list of unsolved problems and the reservations about the unproved nature of the structure, the final paragraph leaves little doubt that the authors thought they had a good idea.

How do they stand today?

How have these early papers stood the test of time? It can now be taken as firmly established that DNA usually consists of two chains, wound together and running in opposite directions. The evidence for this statement is so extensive that it would take too long to quote it all here. The fact that normally A pairs with T, and G with C, is also well established but the details were less certain until recently. The G:C pair was never in serious doubt. Watson and I drew this with only two hydrogen bonds but mentioned in our technical paper¹ that three was also a possibility. This was made almost certain by the theoretical arguments of Pauling and Corey² and was confirmed by X-ray structure determinations of single crystals of base pairs. The same technique showed that the A:T (or A:U) pair in single crystals usually did not have the configuration Watson and I suggested. The matter was only finally resolved about a year ago when Rich and his colleagues published two crystal structures; that of GpC paired with itself³ and ApU paired with itself⁴ (the backbone in each case was ribose), both to about 0.9 Å. They show not only the expected configurations for the base pairs but also make it highly likely that, as we claimed, nucleic acid helices are right handed.

In 1953 it was uncertain whether RNA could form a double helix. Watson and I stated that we thought we could not build our model for the B form of DNA with an RNA backbone. The discovery of double-stranded RNA viruses proved, however, that biological RNA too could form a double helix, though with slightly different parameters. The detailed coordinates we had (tentatively) suggested for DNA were soon shown to be incorrect (we had put the backbone at too big a radius) and much more accurate coordinates were provided by Wilkins and his colleagues, using fairly sophisticated methods of handling their much improved X-ray data. The general correctness of this work has been strongly supported recently by the single-crystal studies, mentioned above, of Rich and his coworkers.

Recently, Bram⁵ has put forward evidence that the parameters of a DNA double helix may vary somewhat with base composition, though whether this is a trivial variation or has deep biological implications is at present uncertain. Watson and I were so impressed with the apparent uniformity of the double helix from different biological sources and the regularity of the backbone of our model that we had no hesitation in saying that it "seems likely that the precise sequence of the bases is the code that carries the genetic information", an idea which gave me plenty to think about in the next 10 or 12 years.

Nothing was said about the possibility that the two chains might be melted apart and then annealed together again, correctly lined up. The discovery of this by Marmur and Doty has provided one of the essential tools of molecular biology. I can still remember the excitement I felt when Paul Doty told me about it at breakfast one day in New York in a hotel overlooking Central Park. But in other respects we were almost too far sighted, as witness our remark that recombination would probably depend upon

base pairing. We struggled for several years to produce neat models for this, all to no avail, partly because we accepted copy choice too easily but also because we were trying to invent a mechanism which did not need additional enzymes. This showed a gap in our overall grasp of molecular biology, which can also be glimpsed in our tentative suggestion that DNA synthesis might not need an enzyme, a remark I should certainly not make today except perhaps in the context of the origin of life.

As to DNA replication, our earliest description was mainly schematic. We realised that plain nucleotides were not likely to be the immediate precursor but missed the rather obvious idea that they were nucleoside triphosphates, again a lack of insight into biochemistry. We did suggest the so-called Y mechanism (in the Cold Spring Harbor paper) but did not mention the difficulties due to the direction of synthesis of antiparallel chains, though I frequently emphasised it a few years later. Looking back, I think we deserve some credit for not being inhibited by the difficulty of unwinding which we clearly recognised and for our forthright stand against paranemic (as opposed to plectonemic) coiling. In this instance our grasp of X-ray diffraction was invaluable.

The functions of DNA

It is, of course, somewhat a matter for surprise that DNA synthesis is not fully understood even today. It would take too much space to discuss the complex and rapidly moving field here. Semiconservative replication in many instances is firmly established. The process certainly occurs as if base pairing were taking place, but I have often asked myself what evidence would make it certain that base pairing really occurs rather than some elaborate allosteric mechanism, even though the latter seems unlikely. Perhaps only an X-ray determination of the structure of the polymerase will finally answer the question. Meanwhile the topics of Okazaki fragments, rolling circle models, RNA primers and the exact roles of the various polymerases will keep many people busy. Even at that early period we did at least ask whether the DNA of a chromosome was in one long molecule, though the idea of circular DNA never occurred to us. Nor did we suggest that a virus might have single-stranded DNA. There is however one remark which may turn out to be perspicacious "... we suspect that the most reasonable way to avoid tangling is to have the DNA fold up into a compact bundle as it is formed". As we struggle with the structure of the *E. coli* chromosome and the even more formidable problem of the structure of the chromosomes of higher organisms—probably the major unsolved problem of molecular biology today—it might be worth remembering this tentative suggestion from the distant past.

The other topic we touched on was mutation. This was of the base-substitution type—there is no hint of frameshift mutants. We totally missed the possible role of enzymes in repair although, due to Claud Rupert's early very elegant work on photoreactivation, I later came to realise that DNA is so precious that probably many distinct repair mechanisms would exist. Nowadays one could hardly discuss mutation without considering repair at the same time.

There is no hint in these early papers that nucleic acid might form a complex three-dimensional structure such as we now find in transfer RNA nor even the idea of the hypothetical Gierer loops. Our message was that DNA was simple and alone carried the genetic information. We saw no reason to complicate it till we had to. For the same reason although we must have drawn a G:U pair we attached no importance to it. "Wobble" was still far in the future, but these, it seems to me, are forgivable oversights.

Reactions to the structure

It is really for the historian of science to decide how our structure was received. This is not an easy question to

answer because there was naturally a spectrum of opinion which changed with time. There is no doubt, however, that it had a considerable and immediate impact on an influential group of active scientists. Mainly due to Max Delbrück, copies of the initial three papers were distributed to all those attending the 1953 Cold Spring Harbor Symposium and Watson's talk was added to the programme. A little later I gave a lecture at the Rockefeller which I am told produced considerable interest, partly I think because I mixed an enthusiastic presentation of our ideas with a fairly cool assessment of the experimental evidence, roughly on the lines of the article which appeared in *Scientific American* in October, 1954. Sydney Brenner, who had just finished his PhD, at Oxford under Hinshelwood, appointed himself, in the summer of 1954, as our Representative at Cold Spring Harbor and took some pains to get the ideas over to Demerec. It was about this time that Matt Meselson, just moving into biology from physical chemistry, grasped the importance of inventing a new method to tackle the problem of semiconservative replication, a theoretical analysis which led to density gradient centrifugation. But not everyone was convinced. Barry Commoner insisted, with some force, that physicists oversimplified biology, in which he was not completely wrong. Chargaff, when I visited him in the winter of 1953-54, told me (with his customary insight) that while our first paper in *Nature* was interesting, our second paper on the genetic implications was no good at all. I was mildly surprised to find, when, some years later, in 1959, I talked with Fritz Lipmann who had arranged that I should give a series of lectures at the Rockefeller, that he had not really grasped our scheme of DNA replication. (It emerged that he had been talking to Chargaff.) By the end of the lectures, however, when he summed up, he gave a remarkably clear outline of our ideas. Arthur Kornberg has told me that when he began work on DNA replication he did not believe in our mechanism, but his own brilliant experiments soon made him a convert, though always a careful and critical one. It was his work which produced the first good evidence that the two chains run in opposite directions. All in all it seems to me that we got a very fair hearing, better than Avery and certainly a lot better than Mendel.

Not that it was all plain sailing. We were naturally delighted with the work of Meselson and Stahl, and of Herbert Taylor, on semiconservative replication, though I have never thought this the essence of our ideas which lies rather in the base pairing. Seymour Benzer's genetic analysis of the r_{II} locus of phage T4 encouraged us greatly. But we had to live through the claims of Marshak that there was no DNA in *Arbacia* eggs and of a Canadian group that the amount of DNA synthesis in one cell cycle was twice the expected amount. At a later stage Cavaliere claimed that the basic DNA structure had four chains, rather than two, an idea which cropped up again more recently. On the crystallographic side Donohue, whose advice had been crucial to our understanding of base pairing, was a persistent critic of the validity of the later X-ray work, but in recent years he carried it too far, refusing, for example, to admit as evidence the great accumulation of data showing that the two chains are antiparallel. (In 1956, he had rashly published, with Stent, a quite erroneous structure having like-with-like pairing.) I hope the recent papers by Rich, referred to above, have to some extent reduced his doubts, which at times had some justification.

Who might have discovered it?

Then there is the question, what would have happened if Watson and I had not put forward the DNA structure? This is 'iffy' history which I am told is not in good repute with historians, though if a historian cannot give plausible answers to such questions I do not see what historical analysis is

about. If Watson had been killed by a tennis ball I am reasonably sure I would not have solved the structure alone, but who would? Olby⁶ has recently addressed himself to this question. Watson and I always thought that Linus Pauling would be bound to have another shot at the structure once he had seen the King's College X-ray data, but he has recently stated that even though he immediately liked our structure it took him a little time to decide finally that his own was wrong. Without our model he might never have done so. Rosalind Franklin was only two steps away from the solution. She needed to realise that the two chains must run in opposite directions and that the bases, in their correct tautomeric forms, were paired together. She was, however, on the point of leaving King's College and DNA, to work instead on TMV with Bernal. Maurice Wilkins had announced to us, just before he knew of our structure, that he was going to work full time on the problem. Our persistent propaganda for model building had also had its effect (we had previously lent them our jigs to build models but they had not used them) and he was proposing to give it a try. I doubt myself whether the discovery of the structure could have been delayed for more than two or three years.

There is a more general argument, however, recently proposed by Gunther Stent and supported by such a sophisticated thinker as Medawar. This is that if Watson and I had not discovered the structure, instead of being revealed with a flourish it would have trickled out and that its impact would have been far less. For this sort of reason Stent had argued that a scientific discovery is more akin to a work of art than is generally admitted. Style, he argues, is as important as content.

I am not completely convinced by this argument, at least in this case. Rather than believe that Watson and Crick made the DNA structure, I would rather stress that the structure made Watson and Crick. After all, I was almost totally unknown at the time and Watson was regarded, in most circles, as too bright to be really sound. But what I think is overlooked in such arguments is the intrinsic beauty of the DNA double helix. It is the molecule which has style, quite as much as the scientists. The genetic code was not revealed all in one go but it did not lack for impact once it had been pieced together. I doubt if it made all that difference that it was Columbus who discovered America. What mattered much more was that people and money were available to exploit the discovery when it was made. It is this aspect of the history of the DNA structure which I think demands attention, rather than the personal elements in the act of discovery, however interesting they may be as an object lesson (good or bad) to other workers.

My own reactions

I have sometimes been asked whether I had ever contemplated writing my own account of the discovery. In the 1950s I did give a lecture on this subject to a group of historians of science at Cambridge and to a similar group at Oxford. I was able to be rather more scholarly than Watson could allow himself in *The Double Helix*, which is better regarded as a rather vivid fragment of his autobiography, written for a lay audience. As to a book I confess I did get as far as composing a title (*The Loose Screw*) and what I hoped was a catchy opening ("Jim was always clumsy with his hands. One had only to see him peel an orange. . .") but I found I had no stomach to go on. Recently we made a film together about it for undergraduates. Much had to be left out when the film came to be cut but it does to some extent supplement Jim's book. Since Olby's detailed and scholarly account⁶ will soon be available I doubt if there is now much more I can usefully add.

Finally one should perhaps ask the personal question—I am I glad that it happened as it did? I can only answer that

I enjoyed every moment of it, the downs as well as the ups. It certainly helped me in my subsequent propaganda for the genetic code. But to convey my own feelings, I cannot do better than quote from a brilliant and perceptive lecture I heard years ago in Cambridge by the painter John Minton (he later committed suicide) in which he said of his own artistic creations "the important thing is to be there when the picture is painted". And this, it seems to me, is partly a matter of luck and partly good judgement, inspiration and persistent application.

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Molecular basis of biological specificity

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Linus Pauling reviews his work on the molecular basis of biological specificity and remembers his erroneous conception of a three-chain helix structure for DNA in 1952.

DURING the decade 1930-40 I formulated a general theory of the molecular basis of biological specificity, involving the idea that biological specificity results from the interaction of complementary molecular structures, with hydrogen bonds among the most important of the weak intermolecular forces between the interacting molecules. The most striking example of specific biological interactions of this sort is the interaction between the two complementary strands of the DNA molecule in the double helix discovered by Watson and Crick 21 years ago.

Early work

My early work was on the determination of the structure of crystals by the X-ray diffraction technique, the determination of the structure of gas molecules by electron diffraction, and the application of quantum mechanics to physical and chemical problems, especially the structure of molecules and the nature of the chemical bond. In 1929, when Thomas Hunt Morgan came to the California Institute of Technology, bringing with him a number of very able younger biologists, I began to become familiar with biological problems, and to think about possible ways in which biological specificity could be explained in terms of interactions between molecules. I worked on several problems of biological specificity, from the molecular point of view, without success; one of them was the problem of explaining the self sterility of the marine organism *Ciona* (the sea squirt), which was being studied by Morgan. In 1934 the problem of the shape of the oxygen equilibrium curve of haemoglobin attracted my attention. Consideration of the structure of haemoglobin led to the idea that investigation of the magnetic properties of this substance and its derivatives would provide valuable information, and work along these lines, in collaboration with C. D. Coryell and a number of students, was initiated. Alfred E. Mirsky of the Rockefeller Institute for Medical Research, who had been studying haemoglobin for several years, came to Pasadena for a year, and he and I formulated a theory of the structure of native, denatured, and coagulated proteins, based upon the concept that a native protein molecule consists of one polypeptide chain (or of two or more such chains) folded into a uniquely defined configuration, in which

it is held by hydrogen bonds between the peptide nitrogen and oxygen atoms, as well as by other weak forces, with denaturation involving a loss of this well-defined structure¹.

Antigens and antibodies

In 1936, while I was on a short visit to the Rockefeller Institute for Medical Research, Karl Landsteiner asked me how I would explain the observed properties of antibodies and antigens by means of their molecular structure. I thought about this problem during the following years, and consulted Landsteiner about the interpretation of sometimes conflicting experimental results. By 1940 I had formulated a theory of the structure and process of formation of antibodies². This theory was based upon the concept that the specific combining region of an antibody molecule is complementary in structure to a portion of the surface of the antigen, with the antigen-antibody bond resulting from the cooperation of weak forces (electronic Van der Waals forces, electrostatic interaction of charged groups, and hydrogen bonding) between the complementary structures, over an area sufficiently large that the total binding energy could resist the disrupting influence of thermal agitation. Precipitating and agglutinating antibodies were assumed to be bivalent, consisting of a central part, with structure common to all or almost all antibodies produced by the animal, and two end parts, the combining regions, with structure complementary to that of the antigen. (The idea of complementary structures for antibody and antigen was suggested by Breinl and Haurowitz³, Alexandert⁴, and Mudd⁵. There is some intimation of it in the early work of Ehrlich and Bordet.) The complementary combining regions were assumed to be formed by the folding of polypeptide chains in the presence of the antigen, in such a way that the forces of attraction would mould the folding chain into a structure complementary to that of a portion of the antigen, with the folded chain then being held in this configuration by hydrogen bonds and other interactions, even after the antibody has dissociated from the antigen on which the combining group was moulded. Dan Campbell, David Pressman, and a number of other workers in our laboratory carried out experimental studies that verified the valence 2 for precipitating and agglutinating antibodies^{6,7} and that left no doubt that the combining regions of antibodies are complementary in structure to the homologous haptenic groups of the antigen⁸. The fit of the combining region of the antigen to the hapten was shown to be close, better than 20 pm in some cases, and the effects of Van der Waals attraction, electrostatic forces, and hydrogen-bond formation were

separately verified in quantitative hapten-inhibition studies. A satisfactory theoretical explanation of quantitative values of free energy of combination of haptens with antibodies homologous to the *o*-, *m*-, and *p*-azobenzene arsenic acid groups on the basis of known intermolecular interactions was reported in 1945 (ref. 9). For several haptens with various groups substituted in the positions of the azo group in the hapten of the immunising antigen the standard free energy of combination, as given by hapten inhibition constants, was found to be proportional to the calculated Van der Waals interaction with the surrounding antibody, which includes proportionality to the electric polarisability of the group. For groups forming hydrogen bonds the energy of the hydrogen bond (1.5 to 3 kJ mol⁻¹, representing the difference in energy of the hydrogen bond formed by the hapten with antibody and with water) was needed, in addition to the term corresponding to electronic Van der Waals interaction. The effect of electric charge was determined by comparison of haptens closely similar in shape, but with a difference in electric charge: in one case¹⁰ comparison of haptens with either trimethylammonium ion or tertiary butyl group, and in the other case¹¹ with either carboxylate ion or nitro group. In each comparison there was indication of a complementary electric charge in the antibody, close to the charge in the immunising antigen. The magnitude of the effect showed the charge in the antibody to be within 320 pm (first case) or 260 pm (second case) of the minimum distance permitted by the Van der Waals radii of the groups. I think that this work, which was based on earlier work by Landsteiner and his collaborators¹², leaves no doubt that the specificity of antibodies is the result of the complementarity in structure of the combining group and a portion of the surface of the homologous antigen.

Nonbiological specificity

It became evident that nonbiological specificity could also be explained in terms of complementarity. I gave an example in a lecture on analogies between antibodies and simpler chemical substances¹³. "The reaction shown by simple chemical substances that is analogous to that of specific combination of antigen and antibody is the formation of a crystal of a substance from solution. A crystal of a molecular substance is stable because all of the molecules pile themselves into such a configuration that each molecule is surrounded as closely as possible by other molecules—that is, if a molecule were to be removed from the interior of a crystal, the cavity that it would leave would have very nearly the shape of the molecule itself. We can say that the part of a crystal other than a given molecule is very closely complementary to that molecule. Other molecules, with different shape and structure, would not fit into this cavity nearly so well, and in consequence other molecules in general would not be incorporated in a growing crystal. This is the explanation of the astounding chemical process of purification by crystallization—from a very complicated system, such as, for example, grape jelly, containing hundreds of different kinds of molecules, crystals which are nearly chemically pure may be formed, such as crystals of cream of tartar, potassium hydrogen tartrate".

In the same paper it is stated that "although crystallisation is the only simple chemical reaction that shows striking similarity to serological reactions with respect to specificity, there are many physiological phenomena that are similarly specific, and for which the specificity can be given a similar explanation. The specificity of the catalytic activity of enzymes is due to a surface configuration of the enzyme such as to make the enzyme complementary to the substrate molecule or, rather, to the substrate molecule in the strained state that occurs during the catalysed reaction. The specific action of drugs and bactericidal substances have a similar explanation. Even the senses of taste and odour are based

upon molecular configuration rather than upon ordinary chemical properties—a molecule which has the same shape as a camphor molecule will smell like camphor even though it may be quite unrelated to camphor chemically. I am convinced that it will be found in the future, as our understanding of physiological phenomena becomes deeper, that the shapes and sizes of molecules are of just as great significance in determining their physiological behavior as are their internal structure and ordinary chemical properties."

Intermolecular forces in biological processes

In 1940 Max Delbrück and I¹⁴ published a discussion of the intermolecular forces operative in biological processes. P. Jordan had advanced the idea that there exists a quantum-mechanical stabilising interaction that operates preferentially between identical or nearly identical molecules or parts of molecules, and is of great importance for biological processes, including the production of new genes identical with the old ones. Delbrück and I pointed out that the specific quantum-mechanical forces between identical molecules could not be large enough to cause a specific attraction between like molecules under the conditions of excitation and perturbation prevailing in living organisms, and therefore could not be effective in bringing about autocatalytic reactions. We wrote that "It is our opinion that the processes of synthesis and folding of highly complex molecules in the living cell involve, in addition to covalent-bond formation, only the intermolecular interactions of Van der Waals attraction and repulsion, electrostatic interactions, hydrogen-bond formation, etc., which are now rather well understood. These interactions are such as to give stability to a system of two molecules with complementary structures in juxtaposition, rather than of two molecules with necessarily identical structures; we accordingly feel that complementarity should be given primary consideration in the discussion of specific attraction between molecules and the enzymatic synthesis of molecules." We mentioned that "The case might occur in which the two complementary structures happened to be identical; however, in this case also the stability of the complex of two molecules would be due to their complementarity rather than their identity." Some time later¹⁵ I discussed the matter of gene replication in more detail: "I believe that the genes serve as the templates on which are molded the enzymes that are responsible for the chemical characters of the organisms, and that they also serve as templates for the production of replicas of themselves. The detailed mechanism by means of which a gene or a virus molecule produces replicas of itself is not yet known. In general the use of a gene or virus as a template would lead to the formation of a molecule not with identical structure but with complementary structure. It might happen, of course, that a molecule could be at the same time identical with and complementary to the template on which it is molded. However, this case seems to me to be too unlikely to be valid in general, except in the following way. If the structure that serves as a template (the gene or virus molecule) consists of, say, two parts, which are themselves complementary in structure, then each of these parts can serve as the mold for the production of a replica of the other part, and the complex of two complementary parts thus can serve as the mold for the production of duplicates of itself." The same statements were made in the spring of 1948 in lectures in Oxford, Cambridge, London and elsewhere.

The hydrogen bond was recognised by Latimer and Rodebush as an important structural feature more than 50 years ago¹⁶. In their 1920 paper they mentioned that "Mr Huggins of this laboratory in some work as yet unpublished has used the idea of a hydrogen kernel held between two atoms as a theory in regard to certain organic compounds." In 1936 Mirsky and I pointed out the importance of the hydrogen bond in determining the structure of proteins¹. In the same

year Huggins also discussed protein structures in a more detailed way, with hydrogen bonds between the NH and CO groups of the main chains¹⁷. A few years later Huggins described several helical structures for polypeptide chains, with intrachain hydrogen bonds¹⁸. These structures were needlessly restricted to having an integral number of amino acid residues per turn of the chain and, moreover, Huggins did not require the amide groups to be planar, although the planarity of these groups had been recognised since 1932 (ref. 19), and had already been verified by several determinations of the structure of simple peptide crystals in our laboratory. It is unfortunate that Huggins was handicapped by these two erroneous assumptions in his imaginative and otherwise sound attack on the problem of the secondary structure of proteins. The same two erroneous assumptions provided a similar insuperable barrier to the vigorous attack made by Bragg, Kendrew, and Perutz on the same problem²⁰. In the meantime, Corey and other investigators in Pasadena had determined the crystal structures of a number of amino acids and simple peptides, and Corey and I had discovered the alpha helix and the parallel chain and antiparallel chain pleated sheets²¹. The discovery of the alpha helix left no doubt about the importance of helical structures and of hydrogen bonds in determining the secondary structures of proteins.

Nucleic acids

I had been interested in the nucleic acids since 1933, when Sherman and I calculated the resonance energy of guanine and other purines²². My colleague Robert B. Corey had made some X-ray diffraction photographs of fibres of nucleic acid, which were, however, of somewhat poorer quality than those published by Astbury and Bell²³. I began work on the problem of interpreting the X-ray photographs on November 26, 1952; on the preceding day I had attended a seminar in biology in the California Institute of Technology, at which Professor Robley Williams of University of California, Berkeley, showed a slide of an electron microscope photograph of molecules of sodium ribonucleate. He said that the small fibrils had a diameter of about 1.5 nm, and that they were apparently cylindrical, in that only one diameter was shown. The X-ray photographs indicated an identity distance along the axis of the molecule of 340 pm, and, with the measured density of RNA, about 1.62 g cm⁻³, it was indicated that the fibres contain two or three molecules, probably helices twisted about one another. The value of the spacing of the principal equatorial X-ray reflection had been shown to decrease with decreasing amount of hydration of the fibres, with a minimum value of 1.62 nm. I assumed this value to correspond to essentially anhydrous nucleic acid, and, using the density, I calculated the number of polynucleotide chains per unit to be exactly three. This result surprised me, because I had expected the value 2 if the nucleic acid fibres really represented genes. I decided, however, that probably the fibres were artefacts, produced by the process of extraction from cells and the subsequent stretching. During the next month I strove to find a way of arranging the polynucleotide chains in a triple helix, and was successful, although the structure was described as "an extraordinarily tight one, with little opportunity for change in positions of the atoms". The paper in which this structure was described was communicated to the *Proceedings of the National Academy of Sciences* on December 31, 1952, and a copy of the manuscript was sent to Watson and Crick²⁴.

In hindsight, it is evident that I made a mistake on November 26, 1952 in having decided to study the triple helix rather than the double helix. It is likely that the fibres giving the equatorial spacing 1.62 nm contained some water, and also had a density less than 1.62 g cm⁻³. The diameter 1.5 nm observed by Williams for nucleic acid molecules corresponds, with an assumed density of 1.6 g cm⁻³ and unit translation 340 pm along the molecular axis, to two molecules in a

helical structure (calculated diameter 1.6 nm) rather than to three (1.9 nm). I am now astonished that I began work on the triple helix structure, rather than on the double helix. I had not forgotten that Delbrück and I had suggested that the gene might consist of two complementary molecules, but for some reason, not clear to me now, the triple chain structure apparently appealed to me, possibly because the assumption of a three-fold axis simplified the search for an acceptable structure.

I cannot say what would have happened if I had made the other assumption, that of a double helix, on November 26, or if I had succeeded in getting access to the diffraction photographs of DNA that had been made by Wilkins. There is a chance that I would have thought of the Watson-Crick structure during the next few weeks. I knew that the purines and pyrimidines were present in nucleic acid in equal amounts, but I had not drawn the reasonable conclusion about purine-pyrimidine pairs. I knew about hydrogen bonding by purines and pyrimidines. Nevertheless, I myself think that the chance is rather small that I would have thought of the double helix in 1952, before Watson and Crick made their great discovery. After all, I had spent part of the summer of 1937 in a search for ways of folding polypeptide chains, with planar amide groups of the correct dimensions and with hydrogen bonds between the CO and NH groups of residues separated by some distance along the chain, in such a way as to account for the X-ray diffraction photographs of alpha keratin, but without success. There was no reason why the alpha helix should not have been discovered then, rather than 11 years later, when it was discovered after a few hours of work. There is no doubt that even rather simple ideas sometimes are very elusive.

It is my opinion that if Watson and Crick had not carried on their persistent effort, and had not had the benefit of advice about the structures of the nitrogen bases and hydrogen bonds from Jerry Donohue and information from the excellent X-ray diffraction photographs of Wilkins, the discovery of the double helix, which has led to such great developments in molecular biology, might well have been delayed for several years.

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Molecular biology in a living cell

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Dr Gurdon discusses the introduction of purified macromolecules, such as nucleic acids and proteins, into living cells in controlled amounts and in such a way that they function normally.

MUCH of the success of molecular biology during the past 20 years has stemmed from the development of methods for purifying macromolecules. Initially this opened the way towards a detailed description of the composition and three-dimensional structure of macromolecules. Subsequently, it enabled the function of identified cell components such as nucleic acid polymerases, messenger RNA and initiation factors, to be determined using cell-free systems which synthesise DNA, RNA or protein. There are however certain questions about the function and behaviour of macromolecules which are unlikely to be answered by a knowledge of their structure and of their behaviour in cell-free systems. These are questions, moreover, which could be answered if it were possible to introduce purified macromolecules into living cells in significant amounts and in such a way that they function normally. This article summarises what is known of the fate of purified macromolecules inserted into living cells, and outlines some of the present and future uses for this particular type of molecular biology.

Macromolecules do not enter cells at all readily under normal conditions¹ (see Fig. 1a). There are exceptions to this rule, such as the passage of some hormones into responsive cells, the uptake of RNA by macrophages and the events involved in virus infection, but in these cases cells possess special receptors or mechanisms for the selective uptake of a restricted range of molecules. Even when agents are used which increase incorporation, such as DEAE dextran^{2,3}, purified molecules, such as RNAs, are susceptible to breakdown if added to undefined media of the kind preferred by growing cells. Only a small proportion of macromolecules in the medium enter a cell over many hours and those which do may be partly degraded. A different approach to the problem is to inject a solution of macromolecules directly into a living cell. By this means, a known number of purified macromolecules can be inserted directly into a living cell without significant contact with the external medium. The manipulations required⁴ are relatively undemanding so long as large cells are used as recipients. Here special emphasis is given to injection experiments with amphibian eggs and oocytes; these cells are especially suitable for such experiments on account of their large size, which permits quantitative work, and their developmental potentiality, which, in the case of eggs, ensures that injected molecules are distributed to different kinds of specialised cells.

Experiments involving the injection of macromolecules into living cells fall into two categories. The first includes attempts to validate injection experiments by proving that introduced macromolecules are accepted in a normal and functional way by the recipient cells. The second involves the application of this technique to specific questions about the characteristics of the injected molecules and the recipient cells.

Validity of injection experiments

We need to know that injected molecules are not rapidly

broken down or eliminated from cells, that they are not toxic in moderate amounts, that they are distributed in cells in their characteristic way, and that they function normally. The most exacting test of these requirements is provided by following the fate of injected macromolecules which have been purified away from other molecules with which they are always associated in cells. As far as is known such molecules as messenger RNA and DNA never exist in cells free and uncomplexed with proteins. Another kind of sensitive test

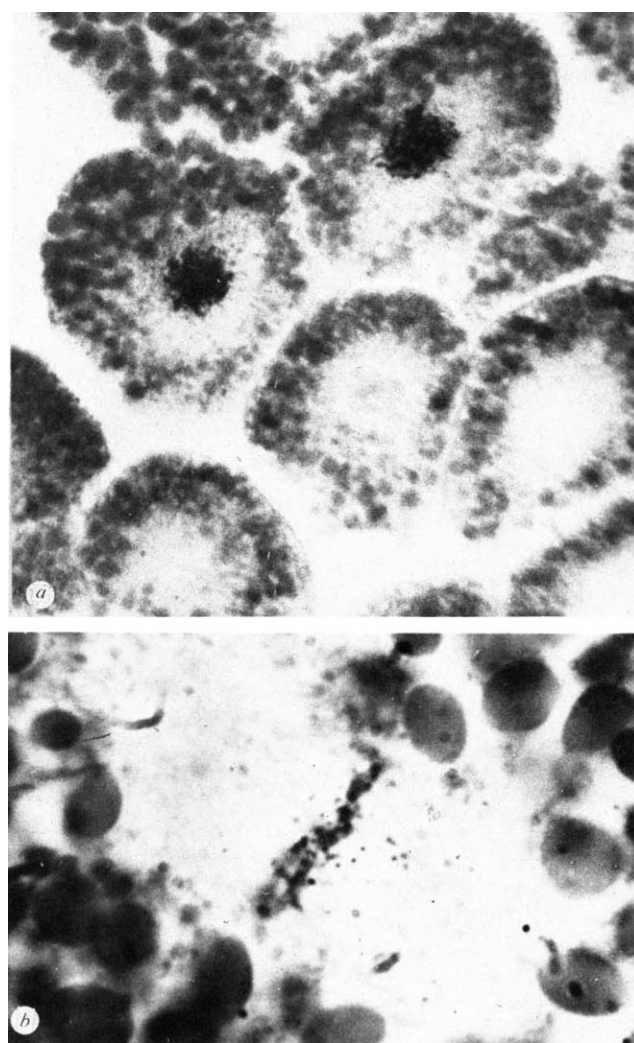


FIG. 1 Autoradiographs of sections of blastulae grown from eggs in which one of the blastomeres was injected at the four-cell stage with *Xenopus* erythrocyte histones labelled with ¹²⁵I. *a*, Two cleavage cells, which are presumed to be daughters of the same parent cell, and which have intensely labelled nuclei. The neighbouring cells, presumed to have been derived from uninjected blastomeres, are completely unlabelled. Evidently labelled histones do not pass freely across cell walls. *b*, Autoradiographic grains over chromosomes on a metaphase spindle. Some of the injected ¹²⁵I-histones appear to have become associated with chromosomes. These are the results of unpublished experiments with W. M. Bonner.

TABLE 1 Tolerance of fertilised *Xenopus* eggs to injected macromolecules

Injected molecules			Survival§, ¶		Relative increase§,		
Type	No. per egg	Weight per egg†	% injected eggs surviving to Blastula (st. 9)	% injected eggs surviving to Tadpole (st. 40)	Injected molecules as a proportion of normal content of equivalent molecules Fertilised egg (st. 1)	Blastula (st. 9)	Tadpole (st. 40)
Proteins*							
Total histones (<i>Xenopus</i>)	5 × 10 ¹² 5 × 10 ¹¹	100 ng 10 ng	Mostly abnormal 85%	4% 52%	0.2 (relative to total histone)	0.05	0.005
Histone f2a2 (calf thymus)	5 × 10 ¹¹	10 ng	94%	67%	1.0 (relative to histone f2a2)	0.25	0.025
Myoglobin (whale)	5 × 10 ¹¹	20 ng	83%	72%	Myoglobin probably absent from eggs and blastulae		Probably low in tadpoles
RNA†							
Globin mRNA (rabbit or mouse)	5 × 10 ¹⁰	18 ng	82%	41%	1.0 (relative to total poly (A)-mRNA)	1.0	0.2
DNA							
Total chromosomal (native calf thymus) (10 ⁷ daltons)	2 × 10 ⁸ 5 × 10 ⁷	4 ng 1 ng	Mostly abnormal 84%	2% 22%	100 (relative to total nuclear DNA)	0.005	0.0004
Polyoma virus (form I native closed circles)	5 × 10 ⁶	0.02 ng	88%	40%			
Injection medium Control	—	—	90%	86%	—	—	—

This table shows the upper limits of tolerance of fertilised eggs, which are more sensitive than oocytes, to introduced macromolecules. Survival to the tadpole stage is a sensitive test of toxicity (see below). Some frog species have larger eggs with greater tolerances.

* Unpublished experiments of W. M. Bonner and J. B. G.

† Refs 8 and 22.

‡ Each sample was injected in a volume of 20 nl per egg.

§ For stage numbers see ref. 43.

¶ Up to 5% survival to tadpoles may be attributed to eggs in which excessive leakage has occurred, and which are not therefore a proper test of toxicity. Survivals of 75% or more are not distinguishable from total nontoxicity, because some eggs are congenitally abnormal.

|| Values for the normal content of proteins and mRNA are approximate. The histone content of cleavage embryos is certainly in considerable excess of their nuclear DNA content but may be smaller than the values used to make these calculations (see ref. 27). Eggs contain ~30 ng of poly(A)-containing RNA⁴⁴, but only ~2% of this is being translated on polysomes (H. R. Woodland, unpublished). Relative to fertilised eggs blastulae contain twice as much, and tadpoles about 20 times as much, polysome-associated mRNA (H. R. Woodland, unpublished). Leakage and breakdown (see text) have not been allowed for in comparing the amounts of injected and endogenous molecules.

is provided by following the fate of injected molecules which normally have a characteristic intracellular distribution; examples of these are histones which are synthesised in cell cytoplasm but which very rapidly enter and accumulate in the nucleus⁵.

The leakage or rapid breakdown of injected molecules has been tested by following the fate of ³H-labelled DNA or ¹²⁵I-labelled histones. The disappearance of acid-insoluble label often amounts to 20–30% within the first few hours after injection, but after this its rate of loss is less than 10% per day. Experiments with globin mRNA (below) suggest that it has a half life of more than one week. The electrophoretic analysis of material extracted from tadpoles 3 days after they were injected, at the egg stage, with ¹²⁵I-histone fractions F2a1 and F2b showed the presence of these two proteins as the major labelled components (W. M. Bonner, unpublished experiments involving the injection of separated fractions of calf thymus histones and unfractionated *Xenopus* erythrocyte histones into oocytes and eggs). Apparently most of the injected proteins and nucleic acids are not rapidly eliminated or broken down.

Toxicity tests have been carried out to determine the numbers of different molecules that can be tolerated by oocytes and eggs. If fertilised eggs, which are much more sensitive than oocytes, can develop into normal tadpoles, the injected molecules are considered nontoxic. Table 1 shows the maximum amounts tolerated by a *Xenopus* egg, that is about 1 ng of DNA, and 10–20 ng (~10¹¹ molecules) of proteins or RNA. These numbers of molecules are entirely sufficient for most experimental analyses to be carried out on a small number of eggs (about 10).

The tendency of injected molecules to take up their normal intracellular distribution has been examined by following autoradiographically the fate of ¹²⁵I-histones injected into oocytes and eggs. Labelled histones show a spectacular accumulation in the nucleus of an oocyte; after 12 h they are

about 100 times more concentrated in the nucleus than in the cytoplasm⁶, a situation which can result in the nucleus containing several hundred times more than its normal content of histone associated with DNA. Histones also accumulate strongly in the nuclei of fertilised cleaving eggs (Fig. 1a). The same nuclear accumulation is not shown by nonnuclear proteins such as bovine serum albumin, nor by proteins of the same size range (myoglobin) or charge (lysozyme) as histones (W. M. Bonner, unpublished). The examination of metaphase chromosomes suggests that at least some of the injected histones become associated with chromosomes (Fig. 1b). Separated calf thymus histone fractions give broadly similar results to those just outlined for *Xenopus* erythrocyte histones. Some of the injected mRNA seems also to take up its normal location in injected cells, since globin mRNA becomes incorporated into polysomes (mostly pentasomes)⁷ and its translation is inhibited by cycloheximide⁸. The fate of DNA injected into fertilised eggs is now under investigation, but other work on DNA which enters cultured plant or animal cells has shown that some of the DNA is included in the nucleus^{9,10}, and may be linked to chromosomal DNA^{11,12}.

Evidence for the normal function of injected macromolecules has come from experiments with RNA and DNA, since the function of histones is not known. Results obtained with globin mRNA have shown conclusively that some of the injected molecules can function normally. α and β globin mRNAs of the rabbit, mouse and duck are all translated accurately, as judged by several different types of analysis of oocyte-synthesised haemoglobin, globin and globin peptides^{13–15}. Furthermore, the injected mRNA is used efficiently; appropriate calculations¹⁶ suggest that, on average, one globin molecule is synthesised in oocytes from each mRNA every 2 min. This is about four times less often than would have been the case in rabbit reticulocytes at 19° C. If, however, some of the injected molecules leak out, are broken down, or

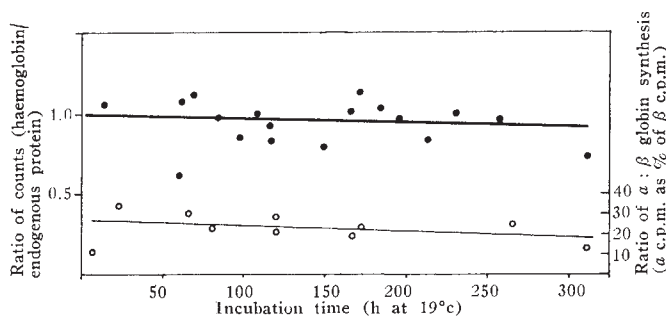


Fig. 2 Stability of globin messenger RNA in *Xenopus* oocytes. Purified mouse globin mRNA (7 ng per oocyte) was injected into several groups of oocytes which were incubated in unlabelled medium. At intervals, a group was transferred to ^3H -histidine-containing medium for 12 h, and then analysed for the ratio of labelled haemoglobin to labelled endogenous proteins (●) and for the ratio of α globin to β globin synthesis (○). Details in ref. 16.

are defective, the efficiency with which some of the injected molecules are translated would be close to what is normal for the cells from which they were prepared.

Evidence for the function of injected DNA has been obtained for replication, though not as yet for transcription. The strongest case for replication of DNA has come from the injection of double-stranded supercoiled polyoma virus DNA into eggs¹⁷. The tests carried out include the demonstration that heavy:light parental molecules, density-labelled with BUdR on one strand, cause the appearance in eggs of light:light double-stranded molecules. ^3H -TdR incorporation was observed into new strands which were complementary, and hydrogen bonded, to the density-labelled parental strands. Since the newly synthesised polyoma DNA was selectively extracted, and purified in alkaline sucrose gradients as 53S molecules, it is evidently present as closed supercoiled circles. The egg cytoplasm has apparently been able to open the parental supercoils, synthesise a copy semiconservatively, separate the newly synthesised molecules, and close them up into circles. It seems that different kinds of vertebrate native DNA will serve as a template for replication in egg cytoplasm¹⁸, but do so to a much lesser extent in oocyte cytoplasm^{19,20}. This difference is important since the behaviour of injected molecules reflects the normal metabolic difference between oocytes and eggs in respect of DNA synthesis, a result which gives confidence in the significance of such experiments.

These results with RNA and DNA allow us to conclude that macromolecules can be purified away from their natural association with other molecules, injected free into the cytoplasm of eggs and oocytes, and still carry out some of their normal functions. These results, together with the evidence presented that injected molecules are not rapidly degraded or toxic, justify attempts to use injection experiments for investigating certain properties of injected molecules and recipient cells.

Specific applications of injection experiments

The following experiments have given results which would not have been predicted; they have been selected to show how the introduction of macromolecules into living cells can provide information not readily obtained by other means. The examples selected concern secondary modification of proteins, message stability, message-specific translational factors, and *in vivo* DNA polymerase assays.

Many kinds of proteins are secondarily modified after synthesis; molecular injection experiments have led to the conclusion that the enzymes involved are surprisingly general. Table 2 shows that frog egg or oocyte cytoplasm can modify correctly a range of proteins which they would never normally encounter. This lack of cell-type specificity of modifying enzyme systems suggests that the existence of modified pro-

teins in a cell is determined by the availability of amino acid sequences on which enzymes act and not by the availability of the enzymes themselves.

The stability of mRNA can vary substantially from one kind of message and cell type to another²¹, and message instability could be important for ensuring a changing population of new proteins as cells differentiate. The introduction of mRNA into a cell which is not synthesising that kind of mRNA provides a very sensitive test of mRNA stability under *in vivo* conditions. As seen in Fig. 2, α and β -globin mRNAs continue to be translated in oocytes for as long as 2 weeks, and without any obvious reduction in efficiency. Comparable experiments have been carried out in fertilised eggs²² and again the injected messages continued to be translated for as long as a week; by this time, over 15 cycles of cell division had been passed through, and the eggs had developed into swimming tadpoles, which were still synthesising rabbit haemoglobin. This remarkable stability of globin mRNA excludes the possibility that there is a general mechanism in oocytes or embryos which degrades all kinds of messages. Any natural message which is short lived in these embryos must evidently have RNase sensitivity coded into it, or must be specially complexed or compartmentalised in the cell.

Specialised cells are believed to contain molecules which enhance the translation of their characteristic messages²³. The effect of these message-specific factors is seen in cell-free systems. The unnatural composition of *in vitro* systems however, could result in a dependence on such factors in a way that would not be true of living cells. The use of injection experiments for investigating the role of message-specific factors and the control of translation in general demands careful quantitative control over the injection of nanolitre volumes of material. That this can be achieved in living cells is seen from the message saturation curves in Fig. 3. The injection of more than 10 ng of globin mRNA saturates the cell, and some component other than mRNA then limits the rate of its translation. Furthermore a message, once established, is displaced only slightly if at all by large amounts of other injected messages¹⁹. This information makes it possible to carry out tests for the existence of message-specific factors by injecting mRNA into an oocyte which has previously been saturated with a different kind of mRNA. If the translation of a second message is unaffected by whether the cell has been previously saturated with the first message, this implies the existence of message-specific factors. Such experiments have been carried out in which globin and encephalomyocarditis (EMC)

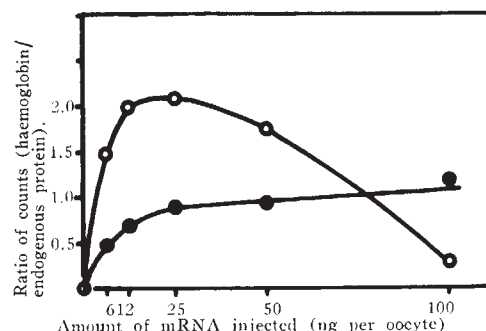


Fig. 3 Message saturation curves obtained by injecting increasing concentrations of mRNA or polysomes (for rabbit globin) into *Xenopus* oocytes. In calculating mRNA concentrations, it has been assumed that globin mRNA is 100% of the repurified 9S RNA of reticulocytes, and is 1.5% of the total RNA of polysomes. These experiments were carried out and analysed according to the procedures described in ref. 49. At least part of the difference in the saturation levels of polysomes (○) and pure mRNA (●) is due to the more efficient translation of α -globin mRNA in polysomes (which were in this case a crude preparation containing haemin)¹⁶. These are unpublished results obtained in collaboration with G. Marbaix.

TABLE 2 Secondary modifications of proteins in *Xenopus* oocytes

Protein	Modification in normal tissue of origin	Cell type in which protein is normally synthesised and modified	Evidence for modification in frog oocytes
α A2 crystallin	Acetylation of N-terminal methionine	Calf lens epithelium	N-acetyl methionine in N-terminal peptide ⁴⁵
Stable proteins of EMC virus	Cleavage of primary polypeptide	Mouse ascites cells	³⁵ S-methionine labelled virion proteins ¹⁷
Protamine	Phosphorylation	Trout testis	Phosphorylated peptides (unpublished work with G. Dixon)
Immunoglobulin light chain	Removal of ~15 amino acids from primary polypeptide	Mouse myeloma cells	Size of labelled light chains ^{46,47}
Collagen	Hydroxylation of proline	Cultured mammalian fibroblasts	Hydroxyproline in labelled protein ⁴⁸

Oocytes were injected with mRNA coding for the proteins specified, and then allowed to incorporate labelled amino acids. Labelled proteins were extracted and analysed. There are indirect, though strong, arguments against the possibility that the message samples contained contaminating messages for the modifying enzymes, as explained in references cited. This list includes all tests for secondary modifications so far completed.

virus RNA compete against each other. The translation of one of these messages is very largely inhibited by the injection of a saturating amount of the other, 24 h before (R. A. Laskey and J. B. G., unpublished). These two quite different messages seem to compete for the same translational components, and seem to be little affected by message-specific factors. This system can be applied to messages of different cell types once these have been purified to a level where they can saturate an oocyte's translational system.

The injection of mRNA into oocytes has been able to demonstrate a specific effect of haemin on the translation of α -globin mRNA. The background to this experiment is that rabbit or mouse α -globin mRNA is translated about five times less efficiently than β -globin mRNA in oocytes and eggs of *Xenopus*, though the α and β -globin mRNAs are translated with about equal efficiencies in mammalian reticulocyte cell-free systems²⁶. If globin mRNA is injected at a subsaturating concentration, the addition of haemin raises the efficiency of α -globin mRNA translation in oocytes so that it is equal to that of β -globin²⁴. This effect shows that haemin behaves as a message-specific translational factor in oocytes, and it may well do so in normal reticulocytes. It is of interest that this specific effect of haemin has not been revealed in cell-free systems²⁵.

Experiments involving the injection of DNA have given the, at first sight surprising, result that active DNA polymerase and other replicative enzymes are present and potentially active in egg cytoplasm, DNA synthesis being stimulated by the injection of DNA into enucleated eggs²⁸. Apparently egg cytoplasm is provided with a large store of DNA replicating machinery which is provided for nuclei during their enormously rapid rate of division during cleavage, when each S phase is completed in 10 min and mitoses occur every 20 min²⁶. It may well be difficult for cleaving embryos, which have small numbers of cells and therefore of genes, to synthesise new messages for all the proteins which are needed in rapidly increasing amounts at this stage. Presumably this is why egg cytoplasm contains a large store not only of active DNA polymerases, but also of histones²⁷, tRNA²⁰, RNA polymerases²⁹, and ribosomes^{28,30}.

Wider application of injection experiments

Molecular biology in living cells is unlikely to be of wide interest if it is restricted for technical reasons to Amphibia which have the advantage of large eggs, but the disadvantage of a long (~1 yr) life cycle. The same technique can however be applied to genetically more felicitous organisms such as *Drosophila* which has an egg nearly 100 times smaller than that of *Xenopus*, and even to the eggs of mice, with a volume 4,000 times smaller than those of *Xenopus*. Yet fertilised mouse eggs can withstand the injection of 20×10^{-12} l of oil droplets³¹ and normal mice have been born from eggs injected with 10^4 molecules of polyoma DNA (J. D. Bromhall, C. F. Graham, J. B. G. and R. A. Laskey, unpublished). It is even possible to inject single cultured cells³²

but with not more than 5×10^{-15} l. Evidently most animal cells can tolerate the injection of at least a few per cent of their own volume, and the introduction of very large numbers of macromolecules not normally present. It is obviously desirable, however, to use amphibian eggs or oocytes whenever possible for such experiments.

Prospects

The experiments described here have been primarily aimed at demonstrating that at least some kinds of purified macromolecules can carry out their normal function after introduction into living cells. What are the long-term prospects for such experiments? Three are worth mentioning. First, an assay system is provided in which the regulatory role of purified macromolecules can be tested in a living cell. It is certain that no cell-free system which might otherwise serve this purpose can reproduce exactly the conditions which exist in a living cell and which are, in any case, not accurately known. Unless the haemin effect referred to above turns out to be exceptional, results obtained from cell-free systems may not give an accurate indication of what would emerge from experiments with living cells.

The second long-term use for introducing macromolecules into living cells is connected with 'rescue' experiments. The injection of purified regulatory molecules, as outlined in the last paragraph, is applicable only when such molecules can be collected in sufficient quantity to be purified. It is very likely, however, that cells make use of regulatory macromolecules which are present only in very small numbers and which have not yet been identified. An attractive procedure for identifying these was devised by Briggs and his colleagues³³, and involves the rescue of embryos which are genetically or experimentally deficient in development. The first successful rescue experiment³⁴ achieved the restoration of germ cell growth and fertility to *Rana pipiens* embryos which had been ultraviolet-irradiated to destroy the germ plasm, a region of egg cytoplasm which is needed for normal gamete differentiation. Subsequently, experiments were carried out with a maternal lethal mutant in the *Axolotl* in which eggs laid by a homozygous mutant (%) always cease development at the late blastula stage (even if fertilised by wild-type sperm³⁵). Some of these lethal embryos were rescued by injecting them as eggs with wild-type egg cytoplasm, so that development proceeded normally for several days beyond the usual stage of arrest³⁶. Some progress has been made in determining the nature of the corrective component^{37,38}. Since developmental arrest is caused in such cases by a single mendelian factor, this design of experiment could lead to the identification of a gene product which plays an essential role in early development. The success of this approach, however, depends heavily on the assumption that the gene product in question can retain biological activity when injected as a purified preparation into egg cytoplasm. The results, summarised here, with injected proteins and nucleic acids certainly give some encouragement for the long-term success of such an

approach. Clearly the isolation of appropriate maternal lethal mutants poses a formidable problem, though some of these have now been isolated in the *Axolotl*³⁹, and *Pleurodeles*⁴⁰; in *Drosophila*, where several suitable mutants are known⁴¹, a rescue experiment has been carried out with *deep orange*⁴².

The third long term use of molecular injection experiments concerns the arrangement of molecules within cells. The asymmetric distribution of molecules has for long been believed to be a primary cause of the first regional differences in developing embryos, and may well be involved in later stages of cell differentiation as well⁴. When, in time, some of these unequally distributed molecules have been identified, it will be possible to deposit samples of them in one region of an egg or other cell type. If the injected molecules remain localised at the site of injection, this will show whether their unequal distribution is causally connected with the divergent cell differentiation that subsequently takes place. If, however, the injected molecules are rearranged within the cell, this will open the way towards an experimental analysis of the cause of the intracellular localisation. The experiences with histones, referred to above, show that at least some kinds of injected proteins can be rapidly redistributed in a cell so as to take up their normal intracellular location.

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Building the Tower of Babble

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In remembering, without nostalgia, the dawn of molecular biology, Dr Chargaff deplores the loss of innocence that science has suffered in his lifetime.

"Two weeks later," Watson writes in his book *The Double Helix*, "Chargaff and I glanced at each other in Paris. A trace of a sardonic smile was all the recognition I got when we passed in the courtyard . . . of the Sorbonne" (page 132). Unfortunately retaining, as I do, only the trivialities of my

past, I remember the incident at the Biochemistry Congress in 1952 and the gawky young figure, so reminiscent of one of the apprentice cobblers out of Nestor's *Lumpazivagabundus*. I felt far from sardonic: I was looking for a toilet; but whatever door I opened, there was a lecture room and the same large portrait of Cardinal Richelieu.

"Sarcastic" or "sardonic" are the attributes usually accorded me in that book. But what I really was, when I first met the fervid pair in Cambridge, was baffled, for here were two people trying to fit the nucleotides into a helix and

worrying about its pitch—it became a double helix, I believe, after I had told them about our results—without bothering to look up the structures of the compounds they wanted to fit together. My dismay before so much unimpeded boldness will be understandable only to those who consider that at this time molecular biology did not yet exist. In the meantime the sciences have learned that it is profitable to transgress their boundaries and to over-reach their competence. Somehow they swallow more than they have bitten off. So perhaps 'laconic' would have been a better attribute, for what I jotted down when I left Cambridge was: "Two pitchmen in search of a helix."

II

It is, however, not of Watson's book that I want to write here; I have done that before¹. What I should like to do is to sketch in a few words how it felt to get into science, and especially into the field of nucleic acids, in those prehistoric times (BWC), before revelations had begun coming down from the mountains; before the 'invisible colleges' had begun to play their disagreeable roles as restrictive guilds; and, for that matter, before *Nature* had begun to sprout those sprightly little *feuilletons*. In addition, I should like to say a few words about the way in which the present scene strikes me.

No one who entered science within the past 30 years or so can imagine how small the scientific establishment then was. The selection process operated mainly through a form of an initial vow of poverty. Apart from industrial employment, important for a few scientific disciplines, such as chemistry, there were few university posts, and they were mostly ill-paid. One of my former chiefs at that time assured me that for him the opportunity of doing research as he pleased was sufficient recompense. (He had, besides, a comfortable private income.)

Science—or at any rate that part of science with which I am familiar—was small; it was cheap; it was wide open. One could still do experiments in the old fashioned sense of the word. Now, everybody is working away at 'projects' the outcome of which must be known in advance, since otherwise the inordinate financial investment could not be justified. Papers, however, continue being written in the old way, as if the discovery had come after the search. The number of very significant scientific discoveries made in the interval between the two world wars was truly enormous. The impulse persisted, or even increased, in the United States up to about 1950 or 1955, and then slackened perceptibly, almost in reverse proportion to the number of new scientists entering the several disciplines. I know of few instances in which the dialectical change of quantity into quality has been so obvious.

Since at that time it cost relatively little to perform scientific experiments, it was always possible and even inviting to venture into fresh areas. The risk was minimal and so, unfortunately, sometimes were the results. But the road to them was always delightful. This changed gradually as a result of several powerful technological advances. The introduction of isotopic labels gave rise to an industry whose products became increasingly more expensive, as the variety of the compounds grew and purity standards declined. The construction of powerful centrifuges and other physical equipment extended immensely the range of the possible, but even more the cost of achieving it. Other advances, especially in chromatography, electrophoresis, and spectrophotometry, contributed more than they took away. Still, it remains that according to my highly unreliable estimate a current paper of mine costs about 20 to 25 times more to produce than an equivalent article 35 years ago, if such things can be weighed and compared. The argument that such calculations are meaningless—for what would we give if we had one more Mozart opera—can be rejected; none of us writes Mozart operas.

III

The small number of scientific workers engaged in research had other consequences. It was easy to open new fields and to go on cultivating them; there was no fear of immediate dispossession as is bound to happen now. There were relatively few symposia, and those that existed were not attended almost exclusively by hungry locusts yearning for fields to invade. Bibliographies were comparatively honest, whereas now entire packages of references are being lifted by a form of transduction, as it were, from one paper to the next; so that if some work gets into the habit of not being quoted, it never will be so again. The break in the continuity of the tradition has, perhaps, been one of the most disastrous effects of the scientific mass society in which we are living now.

The illusion that what is new is true has distorted the very sense of scientific research. The urge to be 'with it' is incompatible with the search for truth about nature—and that is what science is—and where one can say 'this is no longer true,' nothing is true. Some years ago, I heard an eminent colleague declare at a congress: "The results that I reported last year were based on facts that are no longer available"—a form of recantation that should have delighted Galilei without offending the Inquisition. Our current literature is brimming with facts, but many, I am afraid, are no longer available. If the vaunted self-purification of science has broken down some time ago, this is only in part a result of the ever increasing complexity of ever more poorly described experiments. It is even more a consequence of the pressed and driven mood in which research often is performed now: "... in a hurry of waste, and haste, and glare, and gloss, and glitter."²

Two more points will complete this hasty sketch of a Golden Age that never was. As the number of scientists was so small, it was easy for a young man to establish himself. Two or three decent papers, and he was in, for what it was worth. Another consequence of the restricted dimensions of our scientific knowledge at that time, before it was overwhelmed by multiple massive explosions of facts, many of them of the utmost triviality, was that it was still possible to comprehend the essentials of one or even of several sciences. This bucolic security has, I believe, ended: "out of swimmers we have all turned into floaters."³ Or, to put it less metaphorically: "the sciences, like other professions, cannot endure if their practitioners are unable to know more than an ever-smaller portion of what they must know in order to function properly."⁴ Even granting all the present difficulties in acquiring sufficient knowledge, I must say that the extreme dislike, and therefore ignorance, of chemistry I have often encountered among molecular biologists is puzzling. Chemistry is the science of substances; and to the extent that molecular biology deals with substances and not in them, as if they were commodities, a thorough acquaintance with chemistry is advisable.

IV

I should be sorry if I give the impression that I am trying to paint an *aurea aetas*. I grew up in bestial times, and they have become worse. I have written elsewhere of my surprise that such bad times gave rise to so much good science^{5,6}; probably the only activity of the human mind that has, until recently, been in the ascendant. It is, however, not astonishing that in a rotten society even the saints will carry a slight aroma of rottenness.

One of the outstanding curses of my lifetime has been the manipulation of mankind by advertising and propaganda. In the sciences this evil force had for a long time remained unnoticeable, perhaps because expanding capitalism and youthful imperialism had other worries, perhaps because scientists owing to their small number survived unharmed in the crevices of a society that still paid little attention to

them. The first figure of the highest rank that I saw being lifted by the wheels of the publicity machine and turned into a celebrity was Einstein. (Einstein's very interesting correspondence with Max Born and with Mrs Born displays many instances of this process; compare, for instance, the letters of October 7 and 13, 1920, pages 62 and 65.) Freud, who could have been another victim, escaped the glare entirely, being 23 years older. He also benefited from the fact that his discoveries were relatively accessible: an element of impenetrability helps. There may have been before my time another instance of mindless acclaim, namely, in the case of the Curies and the discovery of radium, to judge at any rate from the slightly paranoid image reflected in Strindberg's *Blue Books*; but then one could hardly yet have spoken of the mass media.

When molecular biology appeared on the scene, however, the publicity machines were all in position and it was time for the saturnalia to begin in full force.

V

I should not want to leave the impression that molecular biology began with the double helix. The reason, and even the date, of the appearance of sectarian movements is hard to determine. I have, in another article, attempted to delineate its pedigree which probably had its origin in the discovery of the transforming properties of DNA and the introduction of bacteriophages as objects of biological research^{5,6}. In my opinion, there really was little sense in creating a new science that consisted essentially in the application of chemistry, and to a limited extent of physics, to biology; that is what biochemistry and biophysics stand for.

I remember vividly my first impression when I saw the two notes on DNA that appeared in *Nature* 21 years ago^{8,9}. The tone was certainly unusual: somehow oracular and imperious, almost decalogous. Difficulties, such as the even now not well-understood manner of unwinding the huge bihelical structures under the conditions of the living cell, were brushed aside, in the Mr Fix-it spirit that was later to become so evident in our scientific literature. It was the same spirit that soon after brought us the 'Central Dogma' to which I believe I was the first to register my objection, never having been very fond of gurus with a PhD. I could see that this was the dawn of something new: a sort of normative biology that commanded nature to behave in accordance with the models.

The structural model proposed for DNA in the first note⁸, a helical dyad held together by base pairing, seemed to me not only an aesthetically pleasing solution, but also the most plausible inference from the base-pairing regularities earlier discovered by us in many DNA preparations¹⁰. I was much less in agreement with the scheme for DNA replication proposed in the second note⁹; and even now, 20 years and thousands of experiments later, I cannot say that I am reconciled with it completely, the mechanism of DNA synthesis *in vivo* still being obscure to me.

I do not know whether in 1865, when Kekulé put forward the structural model of benzene, which was to revolutionise organic chemistry, neckties appeared forthwith, embroidered with the pretty hexagons. I should rather doubt it, since at that time mass cretinisation had not yet begun and advertising still was a home industry. At any rate, the publicity carnival that ensued upon the unveiling of the DNA model was probably unique in the history of science. I have given a brief description in my Bertner Foundation Award Lecture¹¹.

VI

Scientific induction is actually the resultant of a parallelogram of rational and irrational forces. That is why in many respects Science is not a science, it is an art. The importance

for scientific research of imagination, of unpredictable conclusions from unexpected analogies, can, therefore, not be overestimated. If all can be declared in advance, then there remains only the dull verification. The greater the reliance on axiomatic constructions, on prescriptive models must be, the more restricted becomes the freedom of the scientific intellect, the narrower is the range of what can be discovered. These are, I am afraid, the conditions under which a great part of molecular biology now operates.

Research always runs the danger of overasserting the truth of its observations, leaving even less space for dialectics to turn around. For me, however, scientific truth consists of what has not yet been disproved: it is at best a dense mosaic of approximations. Therefore the chase is worth so much more than the prey; or, to put it less violently, the road counts more than the destination. Do I hereby propose Sisyphus as the patron saint of the scientist? Not in a general way. What was tragic about the fate of this mythological celebrity was that he was always lifting and losing the same rock; which is, indeed, what many molecular biologists are now doing.

Many of the papers in this field are very competent technically. Since the same procedures are used, regardless of the particular biological system investigated, the results usually confirm each other; and this is taken to prove the unity of nature. When novel apparatus and techniques are introduced, a new set of results is obtained; and this is registered as scientific progress. A pall of monotony has descended on what used to be the liveliest and most attractive of all scientific professions. The noble study of botany, for instance, has been all but banished from many universities. Before, the biological sciences had each their characteristic faces and their distinct spheres of interest into which they drew different types of scientists. Now, when I go through a laboratory, be it of virology or of developmental physiology, there they all sit before the same high-speed centrifuges or scintillation counters producing the same superposable graphs. There has been very little room left for the all-important play of scientific imagination. *Homo ludens* has been overcome by the seriousness of corporate finances.

VII

Our period, just because it is altogether so weak intellectually, is given to extraordinarily strong assertions. Many of the great constructions of our time—existentialism, structuralism, transformational grammar, the central dogma and some other sloganised tenets of molecular biology—have all looked, from their very beginning, somehow shoddy and overblown. There was about them a flavour of not being entirely earned, as of trick images viewed in a mirror. As the mirror clouded over, the images vanished. Much of what they claimed may actually have been true; but they looked like packages much too large for what they contained. One got the impression that it was often the wrapping that produced the particular content; just as there are now packaging artists who wrap entire mountains in plastic flimsy.

This is not the note, however, on which I wish to end. I should like to recall a few names. They are the names of those who had done some of the basic work on nucleic acids and whom I got to know personally, either before or after I left sweeter fields to concern myself with the harsh problems of nucleic acid chemistry. T. B. Johnson, of Yale University, who got me first to America, did some of the most important work on the chemistry of the purines and pyrimidines. Steudel, once one of Kossel's collaborators, I met at the University of Berlin. Alexander Todd showed me through the organic chemistry laboratories when I visited Oxford in 1934. I often saw P. A. Levene at the Rockefeller Institute; his work, especially on the sugar constituents of the nucleic acids, deserves more admiration than it now receives. At

the same institute I sometimes got a glimpse of the great and modest Avery. And there were Gulland and Jordan and J. N. Davidson, Brachet and Caspersson, Bawden and Pirie, Hammarsten and Jorpes, Thannhauser and Gerhard Schmidt, Mirsky and Pollister, and the gentle and courteous Belozersky in Moscow. Zacharias Dische, without whose diphenylamine reaction most of the work on DNA could not have been performed, has for years been a colleague at Columbia University. Many of these men have gone, but the list is, luckily, not entirely sepulchral. They all did their work before the strip-mining of nature had become so prevalent, before researchers had become alienated from the objects of their study. In the tower of forlornness, which the House of Science has become in my time, the inhabitants all speak the same language, but do not understand each other.

Few will be of my opinion, certainly not the one who made fun of me some time ago in a magazine article, saying

that my ideal of a scientist was Louis Pasteur played by Paul Muni. This may be so, though I doubt it. What I do dislike, however, is to see *E. coli* impersonating nature. The difference in talents is really too great.

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- ² Byron, Lord, *Don Juan*, **10**, 26.
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- ⁴ Chargaff, E., *Perspect. Biol. Med.*, **16**, 486 (1973).
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- ⁸ Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737 (1953).
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Molecular biology and metaphysics

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"And now the announcement of Watson and Crick about DNA. This is for me the real proof of the existence of God". (Salvador Dali, 1964).

FRANCIS Crick prefaces his 1966 John Danz Lecture *The Nature of Vitalism*¹ with this quotation from Dali, but mentions neither the surrealist painter nor God in the body of his text. Why, then, does Crick quote Dali? As readers of *The Double Helix* know, James Watson has "never seen Francis Crick in a modest mood". But not even Watson would claim that Crick really believes that they have delivered the final proof for the existence of God. No, my friend Crick finds Dali's statement a tremendous joke and though Dali's intent was surely serious, Crick is making fun of him by according Dali the place of honour under the masthead of an antireligious tract. But I think Dali has sized up the situation quite correctly: the achievements of molecular biology have furnished proof for the existence of God. And that, as I shall try to show here, could be bad news.

To begin, I draw attention to the fact, long appreciated by philosophers, that science rests on metaphysics. That is to say, the attempt of science to explain the world is based on a set of *a priori* beliefs whose validity cannot itself be the subject of scientific enquiry. Now that the once-fashionable philosophy of positivism (which threw metaphysics into the philosophical ashcan) is all but moribund, it should hardly be necessary to belabour this point. But since in *Chance and Necessity*², the best known philosophical book by a molecular biologist, Jacques Monod holds fast to the 17th century empiricist notion of a science free of subjective projections into nature, which Kant had laid to rest nearly 200 years ago, a reminder of the transcendental basis of science may not be out of place.

Platonic doctrine

One of the most fundamental of the *a priori* beliefs that makes science possible is Plato's monistic doctrine of a single principle that not only regulates the course of the sun and the stars but also prescribes to all creatures their proper behaviour. That single principle is God, or his atheistic synonym, Eternal Reason, whose power, according to Isaiah Berlin, "has endowed all things and creatures each with a

specific function; these functions are elements in a single harmonious whole and are intelligible in terms of it alone. . . . This unifying monistic pattern is at the very heart of traditional rationalism, religious and atheistic, metaphysical and scientific, transcendental and naturalistic, which has been characteristic of Western civilization. It is this rock upon which Western beliefs and lives have been founded. . . .³ Thus belief in God is not only not incompatible with rational thought but it is the metaphysical axiom from which it follows that an explanation of the world is accessible to human reason. This accessibility is, in turn, provided for by the existence of God-given Natural Law that governs the orderly operation of the world. Thus a scientist is a man who believes in God, for without this belief it would be futile to try to discover His Laws.

I will document this allegation, which the majority of contemporary scientists surely reject, by quasi-humorous citations from two great scientists, since according to Freud, jokes reveal repressed thought⁴. First, in his Danz Lecture, Francis Crick points out that though the three-dimensional conformation of proteins can, in principle, be worked out from the structure of their component amino acids, the necessary computations are almost prohibitively long. But proteins find their conformations all the same because "Nature's own analogue computer—the system itself—works so fantastically fast. Also she knows the rules more precisely than we do. But we still hope, if not to beat her at her game, at least to understand her". Second, as is well known, Einstein affirmed his unwillingness to accept the epistemological implications of Heisenberg's Uncertainty Principle by saying that "God does not play at dice". By these statements, Crick and Einstein reveal their allegiance to the Platonic doctrine, and Crick probably makes the verbal substitution of a personified 'Nature' for 'God' only to avoid giving the impression that he is a Christian.

The application of the Platonic doctrine is not limited to science, of course, but also provides the foundation for Western ethics. These ethics, held by Judeo-Christians and Western atheists alike, envisage the existence for their own sake, of objectively valid, ultimate values of right and wrong. It is because of the recognition of these God-given (or 'natural') values that we can speak of crimes, or morally justify or condemn any act. But as Berlin points out, there is also another system of ethics, which Berlin terms 'pagan'

and which derives its authority, not from God but from the obvious fact that man is a social animal who lives in communities. In the pagan system there are no ultimate values, no intrinsic rights or wrongs, only communal purpose, and so moral judgements here are relative rather than absolute. Since, our adherence to the Platonic ideal notwithstanding, we cannot ignore the pagan reality, the ensemble of Western aims and values is internally inconsistent. Berlin credits Machiavelli with the discovery of this fact and with showing "that the belief that the correct, objectively valid, solution to the question of how men should live can in principle be discovered is itself in principle, not true". According to Berlin, it is for having split open the Platonic rock upon which Western lives are founded, for pointing out the impossibility of the City of God, rather than for being 'Machiavellian', that Machiavelli has drawn an ecumenical and everlasting hatred from men representing the whole spectrum of Western religious, philosophical and political thought.

Western contradictions

What can be done to resolve this contradiction in Western lives? Admittedly, in the ethical domain one may reasonably doubt whether grafting the lofty ultimate values of Judeo-Christianity on pagan down-to-earth ethics has really been a good thing. But in the scientific domain the notion of Natural Law has evidently been gloriously successful. Particularly since Galileo, modern science has gone a long way in showing that reason can explain the world and that by the understanding thus obtained one can gain mastery over Nature. So how can we give up our Platonic legacy and return to uncorrupted paganism? It is in this light that we must view the triumph of molecular biology over vitalism that both Crick and Monod celebrate in their essays.

Crick has "a strong suspicion that it is the Christians, and the Catholics in particular, who write as vitalists, and it is the agnostics and atheists who are antivitalists". Crick may be right in his suspicions, but I see no philosophical or theological connection between Christianity and vitalism. On the contrary, in so far as vitalism boils down to the proposition that a full explanation of life is beyond the capacity of the human intellect, it subverts the Platonic doctrine. By questioning the accessibility of the world to reason, vitalism raises doubts about the existence of God as the author of Natural Law. Accordingly, the writings of the Jesuit Teilhard de Chardin, a source of inspiration for many latter-day vitalists, were considered heretical by his superiors in the Society of Jesus and suppressed by them. Thus, as long as Schrödinger's query "What is Life?"⁵ had not been answered and as long as science had not been able to account for the basic processes that distinguish living matter from dead, there still seemed a chance that life is not God's handiwork after all. But as Dali recognised, now that molecular biology has shown how life can be explained in terms of ordinary physics and chemistry, further proof has been delivered that God designed the world and saw to it that His plans are comprehensible to man. In other words, by contributing to the validation of the monistic doctrine, Watson and Crick have made it more difficult for Western man to get off his Platonic rock.

The achievements of contemporary biology have helped to bring to light the moral contradictions inherent in the Western ethical system. To appreciate the nature of these contradictions we must consider the concept that is altogether central to that system, namely the soul⁶. Belief in the soul has been as essential for Western ethics as belief in Natural Law has been for Western science. As is well known, the modern formulation of the problem of the soul is due to Descartes, who laid the philosophical foundations for physiology, and thus also for molecular biology, by advancing the fruitful notion that the bodies of humans and animals can be regarded as machines. But since moral principles

obviously do not apply to machines and do apply to humans, humans must be more than automata in human shape. The extra something that makes humans more than automata is the soul. It is from his incorporeal soul that man derives both the freedom of and the responsibility for action, without belief in which there can be no morality. For dealing with the intersection of morals and human biology, nothing has thus far replaced the Cartesian body-soul dualism. Admittedly, as Crick points out, "many scientists believe that the soul is imaginary and that what we call our minds is simply a way of talking about the function of our brains". But in denying their belief in the soul, these scientists, in so far as they are moral beings, merely resemble Molière's Monsieur Jourdain, who did not realise that he was speaking prose. It must be noted that Monod is not one of these scientists, since he devotes a major part of *Chance and Necessity* to showing just why what he calls "the modern soul" is in distress.

Mental illness

Consideration of two examples of a contemporary conflict between biology and morals show that this conflict arises from the impossibility of resolving the body-soul dualism. One of these examples is provided by radical criticism directed against the treatment of the insane, indeed against the very concept of insanity. In an essay, *Mental Illness as a Metaphor*⁷, Thomas S. Szasz, himself a professor of psychiatry, argues that mental illnesses are not genuine diseases and that psychiatry is not a *bona fide* medical specialty. One of the two main arguments put forward by Szasz in support of this claim is that a medical patient can only be a person who voluntarily assumes that role and a physician can only be a person who gives treatment with the consent of his patient. Since according to Szasz, psychiatric treatment is, more often than not, involuntary, insane persons are not really ill and psychiatrists are not really physicians. Thus psychiatric practice maintains not health but law and order, and does so in a manner which is incompatible with the ideals of a free and just society. Szasz' other main argument is that insanity is not attributable to "an abnormality or malfunctioning of [the] body. . . . Strictly speaking. . . . disease and illness can affect only the body. Hence there can be no such thing as mental illness. The term 'mental illness' is a metaphor". Thus, in contrast to Crick's "many scientists" who believe "that what we call our minds is simply a way of talking about the function of our brains", Szasz has recognised the unacceptable moral consequences of that belief. It is a "systematic strategy . . . for stigmatizing and depersonalizing persons". Evidently, Szasz is informed by the notion that the "real" person, the free and responsible agency, is not the body but the incorporeal soul. Thus, to treat insane people as if they were sick is, according to Szasz, to confuse medicine with morals. In other words, the biological reification of the soul, the dissolution of the Cartesian dualism, is incompatible with the maintenance of Western ethics.

Genetic engineering

The second example of a contemporary conflict between biology and morals is provided by the imminent extension of 'genetic engineering' to humans. Though the proponents of a deliberate manipulation of man's genotype intend it in all sincerity to augment human welfare, this prospect is generally viewed with extreme distaste, and not only by the righteous guardians of so-called 'social responsibility' in science. Why should an application of the fruits of biological research of overt philanthropic intent, which is not meant to kill defenceless civilians, exploit subject peoples, or despoil the Earth, nevertheless evoke the spectre of Dr Strangelove? To fathom the root of this problem we may consider a

fantastic facet of genetic engineering which, though it seems taken straight from the pages of Aldous Huxley's *Brave New World*, is actually likely to become a practical reality before long. This is the asexual reproduction, or cloning of humans by transfer of somatic diploid nuclei from a male or female donor to enucleated eggs. Out of such eggs can develop a clone of genetically identical individuals, all of exactly the same hereditary constitution as the donor. This technique will soon make it feasible to abandon the old-fashioned genetic roulette of sexual reproduction and start populating the Earth with identical replicas of carefully chosen, ideal human genotypes. As far as I know, no one has actually come forward to advocate the institution of this programme, even though from a eugenic point of view, it offers tremendous possibilities. Evidently, the idea of cloning humans is morally and aesthetically completely unacceptable. But why is it that whereas it would be fun to have Kant, Beethoven, Isadora Duncan, Einstein, Clark Gable and Marilyn Monroe living on your block, the thought of having hundreds or thousands of their replicas in town is a nightmare? The reason for this horror is the generally shared belief in the uniqueness of the soul. Even though the soul is incorporeal, it is supposed to fit the body, and hence it is not conceivable that a unique soul inhabits each of thousands of identical bodies. In other words, a human clone would not consist of real persons but merely of Cartesian automata in human shape.

To oppose human cloning, however, is to betray the Western dream of the City of God. All utopian visionaries, from Thomas More to Karl Marx, think of their perfect societies as being populated not by men but by angels that embody all of the best and none of the worst human attributes. As long as, due to the vagaries of the sexual reproductive mode, there was not the slightest chance that such angelic populations could actually arise, this seemed like a believable dream, a hope for the future. But now, when advances in molecular and developmental biology have brought asexual generation of homogenous populations selected for angelic properties within practical reach, it suddenly becomes clear that this is not the perfect society that is wanted after all. What is wanted is the impossible: a perfect society made up of a motley collection of imperfect, unique souls, warts and all.

Chinese alternatives

These conflicts are unlikely to be resolved within the context of the Western tradition. What it would take to remove them from Western lives is to abandon belief in God, His ultimate values and His Natural Law. But is it possible to run a civilised society on purely pagan metaphysics? It certainly is, since another great civilisation, the Chinese, has operated for millennia on just that basis. Chinese lives are not founded on the Platonic rock that Machiavelli split open and when viewed in the light of the Chinese tradition, Western scientific atheism is merely old divine wine in new bottles. Just about the time when the Platonic doctrine was formalised in Greece there developed in China the two complementary philosophical-ethical systems of Taoism and Confucianism which have governed life in the Middle Kingdom ever since. The precepts of Confucianism are based on the premise that man is a social creature and that therefore there is virtue in harmonious social relations. These relations are made harmonious not by obedience to universally valid abstract moral principles but by exact adherence to a combination of prescribed etiquette and ritual. Taoism, is a transcendental moral philosophy whose main relevance is for the inner life rather than for social relations. Its precepts are based on the belief that man is part of (non-personalised) Nature and that, therefore, his life must take the path, or *tao*, of natural events. Man, following the *tao*, must abjure all striving,

distrust reason, and attempt to attain a state in which he is as free from desire and sensory experiences as possible. Neither Confucianism nor Taoism invokes God or Eternal Reason as the source of its authority, nor do they posit the existence of Natural Law or ultimate values. Rather, both systems endeavour to provide for man's harmony with his environment.

In my opinion it is highly significant that Chinese attitudes are now coming to exert an ever-growing influence in the West. This influence is no longer confined, as it was only a few years ago, to Zen Buddhists, New Left Maoists, transcendental meditation freaks and other far-out members of the Counterculture. Instead, it has reached the very Pillars of Society. For instance, the recent, mainly middle-class, interest in 'ecology' is a radical departure from the ancient Western aim of dominating Nature. It is nothing less than a Taoist subversion of the hallowed 19th century belief in progress. Similarly, the recent accommodation of the two Superpowers, the United States and the Soviet Union, and the ending of the quarter-century-long Cold War, suddenly abandons, without any profound political change in either country, the righteous reciprocal Crusade to smite the Anti-christ or Enemy of Man. It constitutes a Confucian subversion of the Western romantic ethic of the Nation as the Protector of the True Faith and places harmony above ideology in international relations.

Another important consequence of the Sinification of the West is the recent, and for most old-time scientists quite incomprehensible, loss of faith in the 16th century creed of Francis Bacon that the discoveries of science ameliorate the human condition. More and more people have come to embrace the belief that what it will take to save the world is not to build a better mousetrap but to understand man. But with this development we are reaching the limits of biology: whereas its methods are evidently capable of giving a satisfactory account of human physiology, human psychology does not seem to be accessible to the procedures discovered by Galileo. This conclusion follows from the growing ascendancy of the 'structuralist' over the 'behaviourist' approach to the study of human behaviour. Behaviourism, whose working methods are more or less those of modern science and whose propositions are therefore subject to validation, has thus far produced mainly trivialities. Structuralism, on the other hand, particularly as pioneered by Freud, has offered some deep insights. But structuralist methodology, and its central theoretical axiom that the causal relations governing overt behaviour occur between covert 'deep' mental events whose existence can be inferred only indirectly, renders scientific validation of these insights well-nigh impossible.

Thus the metaphysical implications of modern biology are not quite what my friends make them out to be. It is not the triumph of molecular biology over vitalism that has made belief in God unnecessary. Rather, it is the apparent inaccessibility of the human psyche to scientific study and the prospect that psychoanalysis (which Crick regards as "mental bleeding") may be the best we can do that still leaves open the possibility of abandoning Him. Therefore, not because of but despite "the announcement of Watson and Crick about DNA", the West may still remain free to descend from its Platonic rock and join the East in a pagan One World.

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⁴ Freud, S., *Der Witz und seine Beziehung zum Unbewussten*. (Fischer Taschenbuch Verlag, Frankfurt-am-Main, 1958).

⁵ Schrödinger, E., *What Is Life?* (Cambridge University Press, New York, 1945).

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DNA before Watson—Crick

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"Nucleic acids bulk so large in the composition of cells that it seems certain that these substances are involved in some important biological functions. Nevertheless, with one exception [bacterial transformation], there has been no proof of a specific biological function mediated by one of these polynucleotides; no enzyme, hormone, vitamin, or even vague 'growth substance' has been found to be a nucleic acid."

WITH these words Pollister, Swift and Alfert introduced their work on the metabolism of DNA to a conference on the biochemistry of nucleic acids, held at Oak Ridge in April 1950. Their paper furnishes the historian with clear evidence that by this date DNA was receiving serious consideration as a major component of the hereditary code-script, quite independently of the work on the biology of bacteriophages and on the base composition of DNAs. When Watson and Crick turned to the structure of DNA they knew it was a macromolecular compound, a major constituent of the sperm nucleus, phage head, and chromosome. They could not but be aware of the association between the staining properties of chromatin and of DNA. Only extreme ignorance would have prevented them from knowing of the correlation between chromosome duplication and the synthesis of DNA. Add to these facts the proven identity of the transforming principle with DNA, and one is presented with compelling reasons for identifying DNA with the genetic substance in October 1951 when Watson came to Cambridge and began his collaboration with Crick. Or is the situation only seen as compelling with the aid of hindsight? We must recall that the earlier decision against the genetic role of DNA had to be overcome. It was not so easy in the 1940s as it may seem now to transfer the onus for proof of genetic function from DNA to protein. On what grounds had the candidacy of DNA been rejected and how was the evidence, so damaging to DNA, undermined?

Looking for the hereditary substance

At the end of his life the Swiss physiologist, Fritz Miescher, arrived at the figure of 80% for the nucleoprotein content of the sperm head. His results were reinterpreted by Ostwald Schmiederberg who gave the figure of 96% (ref. 2). Those who accepted that hereditary transmission is due to chemical compounds were therefore anxious to discover in nucleic acids and protamines evidence of chemical differences. They looked for metabolic stability and for continuity from one cell generation to another, but they did not find it! Miescher doubted the importance of nucleoprotein. He had high hopes of a mysterious iron-containing compound which he called "karyogen" supposedly located in the centre of the sperm head, where it was wrapped around with nucleoprotein. His successors, Burian, Mathew, Boveri, Loeb and Strasburger were by no means convinced that either nucleic acid or protamine constituted the hereditary substance. Even the American cytologist, E. B. Wilson, often cited as a supporter of the DNA theory of the gene, in fact identified the genetic substance as 'nuclein', a chemical

compound of nucleic acid and protein. Some authorities regarded mere chemical compounds inadequate for hereditary transmission and invoked 'higher' morphological structures. In this they were supported by the supposed discovery of a cycle of loss and recharging of the chromosomes with nucleic acid. When the cell divided, the chromosomes became short and fat; they took up basic stains avidly and showed up clearly under the microscope. After division these same chromosomes elongated and their power to attract basic stains weakened. The staining substance—chromatin—or DNA, was not, it seemed, the stable continuing genetic material. This must be "achromatin", possibly the protein fraction of the chromosome.

In the sperm head, protein was present in the form of rather specialised compounds known as "protamines". They were found to be much simpler in their composition than any other class of proteins, some having as much as 87% by weight of one amino acid, arginine. Moreover, no continuity could be traced between them and the histone-type of protein of normal body cells. Yet this did not dissuade some biochemists from seeking genetic specificity in the protamines. Thus in 1935 Waldschmidt-Leitz saw the structural comparisons of protamines as preparing "the ground for a chemical investigation of problems of heredity"³. In 1950 K. Felix in Strasbourg was applying paper chromatography to the protamines to determine the quantities of the six mono-amino acids which made up one tenth by weight of the fish protamine, clupein⁴. In 1952 his study of the breakdown products of clupein gave over one-third in the form of four arginine residues strung together, surely reason enough to pass some adverse comment on the potential variety of protamine molecules? No such remark was made⁵.

Others, doubting the potential of both nucleic acids and protamines, searched for other compounds in nuclei. Edgar and Ellen Stedman found only 70% of the sperm head in the form of nucleoprotein; the remaining 30% they attributed to proteins higher than the protamines and histones, which they called 'chromosomin'. This, they claimed was a group of proteins, the amino acid content of which differed from species to species. Now they could overcome the failure of chemistry to provide the required chemical differences by identifying the hereditary materials with chromosomin. Nucleoprotein was no more than a constituent of nuclear sap! To be sure it was condensed on the chromosomes dur-

TABLE 1 Base analyses of nucleic acids from 1902 to 1950

Date	Molar ratios				Authors	Nucleic Acid
	A	G	C	T/U		
1902	1.0(?)	0.98	—	1.17	Osborne and Harris	RNA
1905	0.47	0.23	1.35	1.0	Levene	DNA
1906	1.30	0.98	0.63	1.09	Steudel	DNA
1908	0.98	0.98	1.02	1.02	Levene and Mandel	
1909	1.0	1.3	1.7	?	Levene	RNA
1947	0.8–0.9	1.0	1.1–1.2	1.0	Gulland	DNA
1948	0.8	2.0	1.0	0.25	Chargaff	RNA
1949	1.6	1.3	1.0	1.5	Chargaff	DNA
1950	0.29	0.18	0.18	0.31	Chargaff	DNA

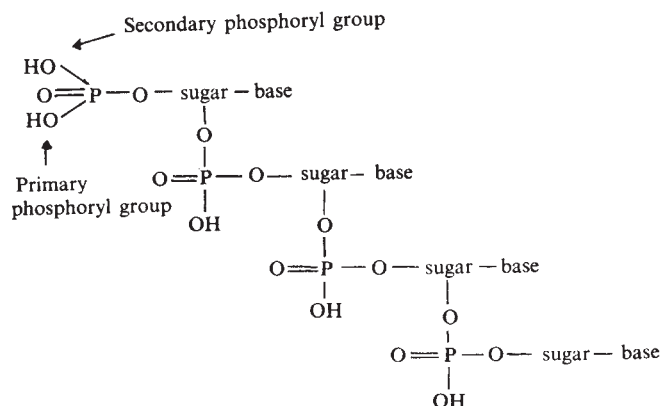


FIG. 1 The tetranucleotide according to Levene.

ing certain phases in the nuclear cycle—as in the sperm head—but this was a transitory state of affairs⁶.

In 1946 Mirsky and Pollister applied their salt extraction technique to trout sperm. The nucleoprotein content of the sperm head came to 91%, leaving 9% of what they called a 'residual protein'. This was distinct from protamine, and like chromosomin, it seemed a promising candidate for the hereditary substance. Not until 1951 did Felix put his authority clearly behind the conclusion that the head of the sperm contains only nucleoprotamine⁸. This search for a residual protein indicates that DNA itself was unacceptable as the genetic material. Although the achromatin hypothesis played a part in achieving this situation it was the tetranucleotide hypothesis which increasingly threw DNA into the shade.

The tetranucleotide hypothesis (or theory as Levene called it) asserted that DNA and RNA had a molecular weight of about 1,300, and contained one of each of the four bases (adenine, guanine, cytosine and thymine in DNA; adenine, guanine, cytosine and uracil in RNA). Because these bases were found to be present in the form of nucleotides (sugar-phosphate + base) the molecule was called a tetranucleotide. Early analyses had shown there to be four phosphorus atoms per molecule, and when four bases were discovered in DNA it was natural to hope to find them in equimolar proportions. The fact that base analyses (see Table 1) deviated by as much as 54% from expectation, and were not always complete, did not trouble these investigators. They had a difficult task on their hands; powerful methods were needed to split the molecule, and bases were easily lost or altered. Osborne and Harris began the tradition of equimolar proportions; Steudel extended it and Levene established it. In the process he twice quoted the figures which Steudel had predicted from the tetranucleotide as his experimental results! Since Levene identified esters of the pyrimidines with two phosphoryl groups attached to them in the breakdown products of DNA he developed an argument for the alternating sequence—pyrimidine, purine, pyrimidine, purine—and rejected any other arrangement. Arguments such as this depended, of course, on the assumption that DNA was a small molecule.

Yet the tetranucleotide hypothesis was not swept away once the macromolecular nature of DNA became established in the 1940s. Doubtless this was to a large extent a case of the entrenchment of a theory. In part it must also have been due to the great success of the theory, for it represented the conclusion of Levene's brilliant work on the constitution of nucleic acids. Out of a confusion of erroneous and vague ideas he had extricated a clear concept—the equimolar proportions of the bases—and built upon it the model of the nucleotide which by sugar-phosphate linkage gave the same chain structure that we accept today (Fig. 1). This hypothesis was, then, a remarkable case of a paradigm which started off as a work-

ing hypothesis, gained explanatory power when associated with the nucleotides, and became so rooted in the chemical thought of nucleic acid chemistry that a revolution was required to overthrow it.

Early estimates of the molecular weight of DNA were based on percentage composition, but although this yielded only the minimum value, it was universally taken to be the true molecular weight—about 1,300. In the nineteenth century Miescher had shown DNA to be a slowly diffusing compound, and Neumann had perceived the distinction between an extract of DNA which readily formed a gel and one which did not. The latter, he thought, resulted from the depolymerisation of the former. But as Fig. 2 shows it was not until 1938 that the macromolecular nature of DNA was demonstrated by Caspersson and the Hammarstens⁹. Levene then obtained crude extracts of the depolymerising enzyme, DNase, and confirmed the Swedes' work. Did he then denounce the tetranucleotide hypothesis?—No! In 1931 he had conceded the possibility that DNA might be a multiple of the tetranucleotide, and in 1938 he was still considering the possibility of depolymerising the macromolecule right down to "a single tetranucleotide"^{10,11}. Nor was he alone in this. The great German nucleic acid chemist, Robert Feulgen, was also convinced that depolymerisation yielded a product with the tetranucleotide structure¹². Even Gulland accepted the polytetranucleotide as a working hypothesis in 1944, but thought better of it a year later and introduced the 'statistical tetranucleotide' to describe a polynucleotide with base composition equal to that for a tetranucleotide, but with a 'random' sequence^{13,14}.

The clearest evidence for the tetranucleotide, it had seemed, was the supposed existence of one secondary phosphoryl group for every four primary groups (Fig. 1). Only by assuming a four-membered chain could the ratio of 1:4 be explained. If DNA was a very long chain this old titration data of Levene's school must have been wrong, or the DNA studied must have been degraded. Gulland and his colleagues at Nottingham repeated these electrometric titrations and found an "almost entire lack of secondary phosphoryl dissociation"¹⁵. This work was published in a symposium of the Society for Experimental Biology in 1947. It was widely read with the result that attempts were made to improve extractive procedures and prevent the action of DNase. This in turn yielded less degraded DNA from which it was possible to obtain good X-ray diffraction patterns.

Bacterial transformation by DNA

The overriding claim of the proteins to be the hereditary substance rested on the demonstration of their biological specificity in enzymes, antigens, hormones, and viruses. The discovery of nucleic acid in the latter did not alter the situation, since no differences between the nucleic acids of different virus strains could be detected. Therefore, the claim of Avery, MacLeod and McCarty in 1944¹⁶ that DNA can cause a specific heritable alteration to a bacterium found no support from experiments in any other quarter. Nevertheless, this work was not neglected, unknown, undiscussed. On the contrary it sparked off several important researches. These led to the discovery of base ratios and the Boivin—Vendrelly rule.

The first to claim that bacterial transformation was achieved by DNA in another bacterium was the French scientist, André Boivin. Although subsequent attempts to repeat his work failed for many years (until 1972), many scientists were impressed by his work. It led him to seek for features in the metabolism of DNA that one would expect of the hereditary material—that germ cells should have half as much DNA as the body cells since their chromosome number is halved. In 1948 Boivin, with his colleagues, the Vendrellys, was able to announce a halving from 6.5×10^{-6} daltons to 3.4×10^{-6}

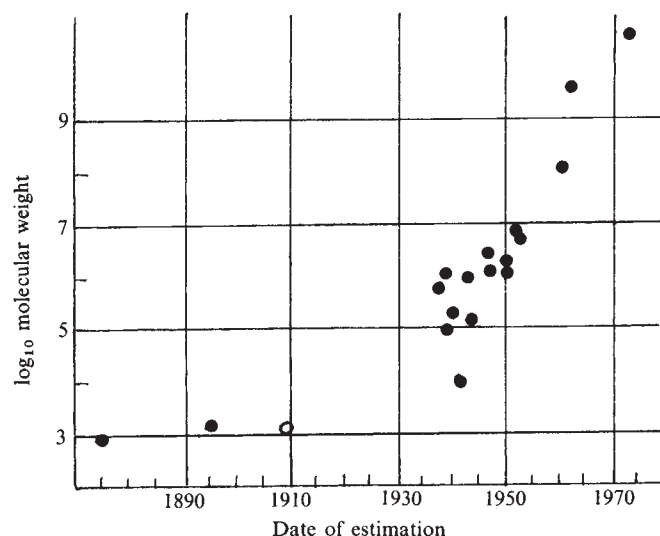


FIG. 2 Molecular weight estimates of DNA. The highest value comes from *Drosophila* DNA; see *Chromosoma*, 48, 1 (1973).

daltons (ref. 17). The significance of this halving rule (the Boivin-Vendrelly rule) was brought out by parallel studies of the metabolic turnover of DNA.

Since 1943 the studies of radioactive incorporation of ^{32}P by Hevesy and his colleagues in Sweden had made plain the contrast between DNA and other cellular compounds, including RNA¹⁸. Its turnover was but a fraction of the others. Also N. J. Davidson, at St. Thomas' Hospital, London, tried starving rats of protein for two days and observing the effect on the DNA and RNA levels in the liver. Whilst the former was unaffected, the latter dipped¹⁹. Boivin took up Davidson's idea and in 1948 a group in Paris presented more striking results which they claimed justified the following conclusions:

"... the absolute quantity of desoxyribonucleic acid enclosed in each nucleus maintains an unchanging value in the course of the most severe fast ...

The invariability of DNA appears as a natural consequence of the special function which is now attributed to it, that of being the depository of the hereditary characters of the species. As for the variability of the RNA; this is easily explained by the very active role which one is inclined to give it in the process of cellular synthesis" (Brachet and Caspersson)²⁰.

At a time when isotopic studies were revealing the very rapid turnover of metabolites here was DNA maintaining a much slower turnover. When Mirsky and Ris confirmed the Boivin—Vendrelly rule they also showed how very variable were the quantities of their 'residual protein'—8.5% to 29% of the chromosomal mass, whereas DNA only varied between 26% and 39%. Now it became clear that the earlier reports of wide fluctuations in DNA percentage content were artefacts. They reflected changes in the absolute quantities of chromosomal proteins. Of these, histones were the predominant class, and recent work had shown that their composition in different tissues of the same organism varied immensely, the arginine from 19% in red blood corpuscles to 87% in mature sperm. Mazia pointed out that in the sperm cell of fish the histone type protein has deteriorated into a much simpler protamine type.

"The sperm cell has no function other than to serve as a genetic bridge between two generations; its physiological capacities are limited to activities that will facilitate the transmission of a nucleus into the egg. If the basic protein fraction actually degenerates in the one case

where we can prove that a full genetic complement is transmitted by the nucleus, it is not tempting to think of this fraction as embodying the properties of genes"²¹.

As far as the apparent metabolic inactivity of DNA was concerned, Mazia saw this as agreeing with the 'template' conception of the gene. "The logical implication is that the gene need not 'do' anything but that it merely provides a blueprint for syntheses". In conclusion, he named DNA as the most likely candidate for the role of genetic material.

Mazia was not the only cell chemist to be impressed by the work of Avery, Boivin and Vendrelly. Arthur Pollister at Columbia University, Hewson Swift at Chicago and Max Alfert at Berkeley were all stimulated by these pioneers to establish further parallels between the genes and DNA, and when a discussion meeting was held at Oak Ridge in 1950 on current problems in the biochemistry of nucleic acids, Pollister spoke warmly of Boivin and described the work of the Vendrellys. Nor was Sir Cyril Hinshelwood, the student of bacterial metabolism, unaware of the Boivin—Vendrelly rule, and from his laboratory of physical chemistry in Oxford came evidence for the constancy of the DNA content and the variability of the RNA content of bacterial cells grown under a wide range of conditions. After suggesting a nucleic acid code for amino acid sequencing (but not a one-way code) Caldwell and he concluded:

"The relative stability of the desoxy-component, and its constancy per cell, perhaps reflect its biological function, in so far as it is believed to be the principal seat of the unchanging hereditary characters, in contrast with the more changeable ribose nucleic acid component. This appears in part to be responsible for the bulk of the protein synthesis, and will presumably be the seat of the variable and adaptable characters"²².

Like Boivin, Erwin Chargaff, at the College of Physicians and Surgeons, New York, was impressed by Avery's work and decided to look for chemical differences in the base composition of DNAs from different sources. In so doing he overthrew the tetranucleotide hypothesis and provided Watson and Crick with the most significant clue to their model—the 1:1 base ratios. It is noteworthy that this achievement was only made possible by the development of chromatography. Just as nucleic acid research benefited from new techniques of molecular weight determination after they had been first applied to the proteins, so it now benefited from the introduction into protein chemistry of paper chromatography. Working under Chargaff, the Swiss chemist, Ernst Vischer, adapted the technique to nucleic acid decomposition products. Fortunately they worked on strikingly different DNA types—the tubercle bacillus (with high content of guanine and cytosine) and yeast DNA (with high content of adenine and thymine). Despite this contrast there was a remarkable approach to equality in the ratios A/T and G/C as in Table 2. In 1950 *Experientia* carried Chargaff's paper on "Chemical Specificity of Nucleic Acids. . ." In his conclusion he affirmed the existence of "an enormous number of structurally different nucleic acids: a number, certainly much larger than the analytical methods available to us at present can reveal"²³. He went on to calculate the number of possible sequences exhibiting the same base proportions as those of the ox. For a chain of 100 nucleotides it was

TABLE 2 Base ratios obtained by Vischer, Zamenhof and Chargaff in 1949

DNA source	Adenine	Thymine	Guanine	Cytosine
Calf thymus	1.7	1.6	1.2	1.0
Beef spleen	1.6	1.5	1.3	1.0
Yeast	1.8	1.9	1.0	1.0
Tubercle bacillus	1.1	1.0	2.6	2.4

10^{56} , for 2500 the number was 10^{1500} . When he addressed a symposium on cytochemistry in 1950 he started by referring to Schrödinger's concept of the hereditary codescript and he went on to give evidence in support of the association of DNA with it. He drew attention to the extension of Avery's work to *E. coli* and *Haemophilus influenzae*. He described his own results, including the classification of DNA into AT and GC types. These facts show that Avery's work did provide the direct stimulus for the most important development in the chemistry of the nucleic acids of that time. No one could claim that Chargaff failed to emphasize the importance of his work, but he failed to achieve a structural explanation of the base ratios.

DNA comes out on top

By the time Watson and Crick came to study the structure of DNA a revolution in the biochemistry of nucleic acids had swept away the old idea of a nucleic acid cycle; a new technique in analytical chemistry had broken the back of the tetranucleotide hypothesis; and from physical chemistry had come new techniques which spelt out the polymeric nature of DNA. By 1950 the chemical and biological specificity of DNA was emerging. To be sure there were qualifications and doubts, halfway positions—the gene is DNA and protein—and just sheer ignorance of these developments. They took several years to spread, and would have taken longer had not the Watson—Crick model speeded up the process. In the meantime one had to read the right literature or belong to the right magic circle. Watson and Crick, by their own admission, did not at first read the literature of nucleic acid chemistry. Most of their clues came from the Phage Group, which until 1952 was ambivalent over DNA and protein. On the other hand members of the Phage Group were not bothered with chromosin or chromosomin; they knew that the nucleic acid of the phage entered the host cell, and by 1952 they were very doubtful that any protein did. On the other hand they were rather arrogant about chemists, so it was not from the Phage Group that Watson and Crick learned about the base ratios, but from Chargaff

himself, when they met him in Cambridge in 1952. Some nine months after that meeting they published their famous model in *Nature*. The key to the model was the pairing of the bases, and this was done in accordance with the Chargaff rules—adenine with thymine and guanine with cytosine, so that $A/T = G/C = 1$. Now, 21 years later, what a rich harvest has been reaped from that neat idea!

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New directions in molecular biology

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"Much has been written about the philosophical consequences of molecular biology. I think it is now clear what the entire enterprise is about. We are looking at a rather special part of the physical universe which contains special mechanisms none of which conflict at all with the laws of physics."

MOLECULAR biology is nothing more than the search for explanations of the behaviour of living things in terms of the molecules that compose them. It is therefore quite an old subject, older than the discovery of the structure of DNA which Watson and Crick announced in this journal 21 years ago. Biochemistry existed as a major scientific enterprise for many years before 1953 and it had made considerable advances in our understanding of intermediary metabolism and biosynthesis in molecular terms. When the double helix appeared it made very little immediate impact on the biochemical establishment which was largely preoccupied with questions about the transformation of matter and energy in

biological systems. It was also viewed with suspicion by most biologists who felt that the complex phenomena of heredity and development could not possibly be explained by what appeared to them to be an excessively simplistic theory. The strong response to the discovery came from quite a small group who took up the new ideas with great fervour. My memory of the early days is that of a small and rather select evangelical movement which often experienced great difficulties in convincing the disbelieving heathen. Looking back, one can now see that the double helix brought the realisation that information in biological systems could be studied much in the same way as energy and matter. It turned genes into chemical objects whose structure and function could be analysed and understood in terms of biochemical machinery.

How this programme, which was only implicit in 1953, has come to be practically realised is now a matter of history. Everybody knows that it depended on the development of powerful methods for fractionating and detecting large molecules, on advances in physical instrumentation and on the judicious choice of organisms for research and their ingenious

genetic manipulation. There were also two other factors which conditioned the explosive growth of the subject and which are not generally emphasised. The double helix fundamentally changed the image of biology. To most young people of my generation the biology taught in universities was a most unattractive subject. It seemed to consist in learning long dusty lists of Latin names punctuated by cutting up frogs or carrots in long dusty laboratories. DNA changed all of that and turned biology into an exciting, intellectually attractive, subject. As a result, large numbers of extremely talented and clever young men who might otherwise have become physicists were drawn to it and the edifice of molecular biology that we have today owes much to their ingenuity and hard work. The second factor was simply the massive expansion of governmental financial support for science and higher education, particularly in the United States, which provided the material base for the rapid growth of molecular biology. Watson and Crick may have invented it but Uncle Sam certainly fuelled it.

Communication

So much for the past; this article is supposed to be concerned with the future. To try to pick out new directions in molecular biology and to do it in a way acceptable to most people has proved to be an impossible task. One of the consequences of the growth of the subject is the vast literature it has generated which nobody can read in its entirety. I estimate that the leading journals alone produce about 10^5 pages of material per year which, in all scholarly conscience, ought to be studied. Nobody can do this and most molecular biologists tend to be conversant with only a small part of the field and rely on others telling them what is interesting or new elsewhere. The rumour, propagated by word of mouth or telephone, has become the main vehicle of communication. If you want to keep something secret in molecular biology, you publish it but do not talk about it. New directions and new ideas in the subject are often generated by one or a small number of people but they become rapidly communalised by this anonymous process of verbal transmission. Here they are held in a reverberating form until someone does the crucial experiment. When that happens, growth can be explosive; if nothing happens, the idea simply peters out. It is this concentration on the experiment that gives molecular biology its rather distinctive and ruthless character and it probably explains why (with the possible exception of Francis Crick) there are no living theoretical biologists. To write about new trends in molecular biology is to tackle a very tenuous field and I can only do it in a very general way.

Developmental biology

First it is clear that the new parts of molecular biology are just the old parts of biology. There has been an increasingly popular move from the study of prokaryotic microorganisms to work on multicellular eukaryotes. Those problems of higher organisms that are directly amenable to the methods of molecular biology have fallen rapidly beneath the axe; antibodies and hormone and drug receptors are recent examples and the analysis of intracellular organelles such as mitochondria is another noteworthy instance. The problem that has only barely been dented is that of development. It is a very old problem indeed. At the turn of the century Thomas Hunt Morgan left embryology to enter the new field of genetics in the hope that it might cast light on the intractable problems of development. One view of modern molecular biology is that it has completed Morgan's deviation and we are now back with the same unsolved problems. How do the genes specify the complex cellular structures that we find in higher organisms? What controls the temporal sequence of development, the geographical layout of cells and their connections, and specific biochemical differentiation? It is not good enough to answer these questions by saying it is simply a matter of turning some genes on and others

off at the right times. It is true that molecular biology provides numerous detailed precedents for mechanisms by which this can, in principle, be done, but we demand something more than these absolutely true, absolutely vacuous statements.

It is interesting to ask whether the problems of developmental biology could be solved by one insight as penetrating as that of the double helix. This is really asking whether higher organisms have some unique piece of molecular biology that is unknown to us. There is a strong tendency of molecular biologists to use every new molecular device uncovered as a basis for a new theory of development. Antibody molecules are composed of heavy and light polypeptide chains which are themselves specified by stitching together a variable and a common gene sequence. There have been numerous speculations on applying this mechanism to the generation of molecular diversity in developing systems. My impression is that these ideas exist in a kind of limbo, neither accepted nor rejected, and probably not even generally discussed. The attitude is that there is nothing to talk about until someone either proves that this is a more general mechanism and not something specific to the immune system.

The general belief is that developmental biology will not be solved at one stroke but its problems will be partitioned into subproblems each of which could be tackled separately, preferably in a model system. One example is cell movement and growth. Clearly, how cells move in relation to each other and how they send out processes is central to understanding development. It is now well established that the motility in cells is generated by actin and myosin molecules closely related in structure to their counterparts in muscle. This turns out to be true of amoebae as well so that the same phenomena with the same underlying molecular mechanism can be studied in detail in a simpler experimental system. It is likely that many more analogous cases will be found and that the cytoplasmic mechanisms of higher cells which provide the machinery of development will, one by one, be isolated and studied in this way.

Eukaryote chromosome structure

There are two problems which are at a different stage of study and which are of great interest at the present moment. The first is the structure of the chromosomes of eukaryotes; the second, the cell surface. It is by now well known that the DNA of higher organisms presents a strange paradox. In brief, there seems to be too much of it for what we think it ought to be doing. When genetic results are compared with the unique sequence content of the DNA in different organisms, the genetic units turn out to be very large. In *Drosophila*, an average gene contains about 20,000 base pairs which is much larger than the 1,000 base pairs required to code for the average polypeptide chain. This is over and above the problem posed by the presence of an even larger excess of redundant sequences. The chromosomes of higher organisms also differ from those of prokaryotes by their content of specific proteins like histones and their far more complex structure. Many theories have been proposed to explain these observations, in terms of the special structural or control requirements of the cells of higher organisms. Solving the structure of chromatin is a very active field at the present moment and success is likely to throw much light on these problems.

An interesting consequence of this new work is that the number of genes in higher organisms is much smaller than we might have been led to expect from their DNA composition. It suggests that *Drosophila* might have as few as 5,000 genes and the small nematode *C. elegans* perhaps only half that number. Using various methods of maintaining lethals it should be possible to isolate mutants in every gene in such organisms. For each of these mutants, as has already been done in a few instances, one could look at the developmental consequences and, with temperature-sensitive alleles, one

could determine the time of action of the gene. Could we gain a detailed understanding of development from this approach? One view is that this might allow us to deduce the logical structure of the genetic programme by seeing whether there are partitioned subsets of genes which correspond in some interesting way to the phenotype. Questions of this sort are not strictly molecular being concerned more with the software of organisms than with their hardware. In the long run, however, we must find the molecular implementations. This would involve identifying the products of the genes and finding out which cells have them and where and when they work to produce their effects. But to do this requires high resolution protein spectroscopy and this technology only exists in a crude and cumbersome form today. Not many people would be willing to embark on such a programme because of its intrinsic magnitude, and there are also many workers who would object on grounds of principle to this approach. There is in fact a fairly large antigenetic school of molecular biologists who believe that the work should proceed from the biochemistry and not the other way around. The only difficulty is that, except in special cases, nobody knows what biochemistry ought to be done.

Cell surface

This brings me finally to the question of the cell surface. One of those nebulous communally circulating ideas is that the cell surface contains the key to the mechanisms of development. These notions have been generated from many different sources. Some come by extrapolation from cell interactions in the immune system, others from the develop-

ment or regeneration of neural connections, and yet others from the study of the reaggregation properties of embryonic cells. The central thought is that cells or groups of cells carry a specific chemical code on their surfaces and by this code they know themselves and their neighbours. In the pages of this journal there have appeared various baroque forms of this theory in which gene stitching, reverse transcriptase and other pieces of molecular clockwork have been grafted onto the idea of specific chemical labelling of cells. Nobody will deny that cells do have surface proteins and that some of these will be involved in recognising specific chemical signals such as nerve transmitters or hormones. The real question is whether there really exists a system which, by one mechanism or another, can generate 10^6 chemical names and plant these on the right cells. There seems to be no convincing general argument either for or against this view and only time will tell.

Much has been written about the philosophical consequences of molecular biology. I think it is now quite clear what the enterprise is about. We are looking at a rather special part of the physical universe which contains special mechanisms none of which conflict at all with the laws of physics. That there would be new laws of Nature to be found in biological systems was a misjudged view and that hope or fear has just vanished. Our job is simply to find out how these interesting pieces of machinery work, how they get built and how they came to be the way they are. In one sense, the answers already exist and all we have to do is to find out how to look them up in Nature. That is why molecular biology seems to me to be the art of the inevitable.

Rosalind Franklin and the double helix

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A draft manuscript shows how near Rosalind Franklin came to finding the correct structure of DNA.

SOME years ago I gave an account¹ of Rosalind Franklin's contribution to the discovery of the structure of DNA, based on published sources supplemented by references to her notebooks and reports. I pointed out how close Franklin had come in the progress of her work to various features of the structure contained in the correct solution. At the time, however, I did not know of the existence of a draft manuscript which confirms how close she had got to the answer. This only came to light later. I therefore take the opportunity of this 21st Anniversary issue to fill out the record and to highlight a dramatic element in the 'race' for DNA.

In my article I told how the analysis of the B form of DNA in terms of helical diffraction theory, which is given in Franklin's paper with Gosling in *Nature*² on April 25, 1953, can be found in her notebook for the period January to March 1953, that is before the Watson-Crick structure had become known to her. I went on to say, however, that she apparently did not feel convinced enough of the relevance of this analysis to publish it (because she had not solved the A form). This is erroneous. The typescript I have found is dated March 17, 1953 and is clearly a draft of the *Nature* paper. This suggests a different explanation from the one I gave, namely that she was proposing to publish what she knew on the B form, two other papers on the A form having already been submitted (before March 6) to *Acta Crystallographica*. This draft contains all the essentials of the *Nature* paper, and much of the wording is carried over intact. It required only slight modifications to take into account the Watson-Crick structure, news of which reached

King's College on March 18, one day later.⁴ In the final (undated) typescript, there is inserted by hand the sentence "Thus our general ideas are not inconsistent with the model proposed by Watson and Crick in the preceding communication."

In the draft it is deduced that the phosphate groups lie on a helix of diameter 20 Å, that is, on the outside of the molecule, in accordance with the conclusion Franklin had reached earlier on the basis of physicochemical reasoning, including her own work on the water uptake by DNA fibres undergoing the A-B transition. Moreover, on the basis of the intensity distribution in the X-ray pattern, she concludes that there is not one chain in the helix, but probably two, coaxially arranged, and that these are separated by $\frac{3}{8}$ of the period, or 13 Å, along the fibre axis direction. The wording is so couched, however, as to show Franklin had not yet understood that the two chains must run in opposite directions, although she had already observed in her notebook that this must be the case in the crystalline A form (which has a two-fold axis of symmetry). I have already argued that it would not have taken long for her to see the true relationship between the two forms—at the time she was thinking of the A form as an unwound version of the helices in the B state (rather, I imagine, like the β -sheet structure is to the α helix in polypeptides).

This would have brought her to the final and crucial step necessary to the complete solution, base pairing. Franklin had thought from an early stage that the bases must lie on the inside of the molecule and be linked by hydrogen bonds, and she had already formed the notion that the two purines were interchangeable with each other and also the two pyrimidines³. An entry in her notebook shows that she was considering Chargaff's analytical data, though there is nothing

to show that she knew the correct tautomeric forms of the bases. The step from base interchangeability to base pairing is a large one, but the idea would have been essential to fitting the variable parts of the structure, the bases, in to the regularly repeating part, the double helix of phosphate-sugar chains at which she had arrived by March 1953.

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⁴ Olby, R. C., *The Path to the Double Helix* (Macmillan, London, in the press).

Molecular biologists come of age in Aries

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It seems that more molecular biologists are born under Aries than any other sign.

ONE major tenet of astrology is that the 'Sun sign' (the constellation of the zodiac in which the Sun was located at the moment of a person's birth) has a powerful influence on

personality¹. I have compared the birthdays of two very different kinds of biologists—taxonomists and molecular biologists. All names listed under all of the taxonomy terms (cyto-, insect, and plant taxonomy; systematic botany, entomology, ichthyology, and zoology; systematics; taxonomic botany, and taxonomy) and all of the molecular biology terms (molecular biology, biophysics, genetics, pharmacology)

TABLE 1 Relative frequencies of births under the different Sun signs

Sun sign	No.	Taxonomists		No.	Molecular Biologists		Month	General Population	
		%	Index		%	Index		%	Index
Aries	28	8.2	98.2	58	12.3	148.0	March	8.4	101.4
Taurus	30	8.8	105.3	32	6.8	81.6	April	8.1	97.1
Gemini	31	9.1	108.8	39	8.3	99.5	May	8.1	97.5
Cancer	38	11.1	133.3	41	8.7	104.6	June	8.2	98.9
Leo	32	9.4	112.3	32	6.8	81.6	July	8.6	103.7
Virgo	31	9.1	108.8	42	8.9	107.1	Aug	8.7	104.8
Libra	25	7.3	87.7	41	8.7	104.6	Sept	8.8	105.4
Scorpio	18	5.3	63.2	41	8.7	104.6	Oct	8.2	98.4
Sagittarius	27	7.9	94.7	40	8.5	102.0	Nov	8.0	95.6
Capricorn	25	7.3	87.7	33	7.0	84.2	Dec	7.9	94.6
Aquarius	26	7.6	91.2	35	7.4	89.3	Jan	8.3	100.0
Pisces	31	9.1	108.8	36	7.7	91.8	Feb	8.6	102.7

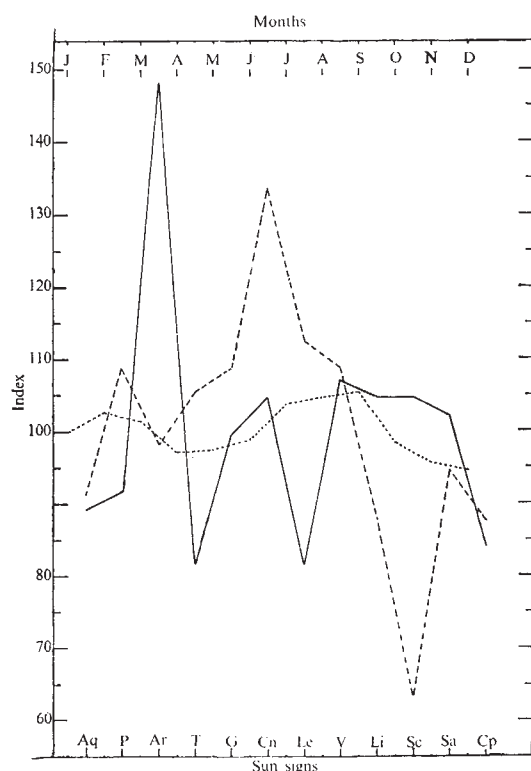


Fig. 1 Relative frequencies of births, expressed as index values, under the different Sun signs for taxonomists (---) and molecular biologists (—); also shown are monthly birth data for the United States in 1934 (· · ·).

were taken from the Discipline Index² of the twelfth edition of *American Men and Women of Science*³. The birthdays were obtained from the main reference portion and the appropriate sun signs were assigned. Names appearing more than once were only counted once. A great deal of variation occurs in the dates for these signs because the sun does not always change signs at midnight, so I used the dates given by Goodman¹. Not all scientists listed had birthdays given. The numbers of persons born under each sign were tallied and the relative frequencies were expressed as an index (each value divided by the average, times 100)⁴ (Table 1, Fig. 1). The monthly birth data for the general population of the United States for 1934 are given for comparison; this is the year closest to the births of most of the scientists now recorded, for which good data are available.

More molecular biologists were born under the sign of Aries than any other sign. More taxonomists were born under the sign of Cancer than any other sign and relatively few were born under Scorpio. Since these peaks do not coincide with the peaks for the general population and are sometimes contrary to them, they are even more remarkable.

¹ Goodman, L., *Linda Goodman's Sun Signs* (Bantam Books, Taplinger Publishing Co., New York, 1968).

² *Discipline Index. American Men and Women of Science. The Physical and Biological Sciences*, twelfth ed. (edit. by Jaques Cattell Press), 135, 231, 304-306, 469, 511-512 (Bowker, New York, 1973).

³ *American Men and Women of Science. The Physical and Biological Sciences*, twelfth ed. (edit. by Jaques Cattell Press) (Bowker, New York; A-C 1:1-1288 (1971), D-G 2:1289-2392 (1972), H-K 3:2399-3496 (1972), L-O 4:3497-4730 (1972), P-Sr 5:4731-6044 (1972), St-Z 6:6045-7184 (1973)).

⁴ Rosenberg, H. M., *Vital Health Statistics*, **21**(9), 1-59 (1966).

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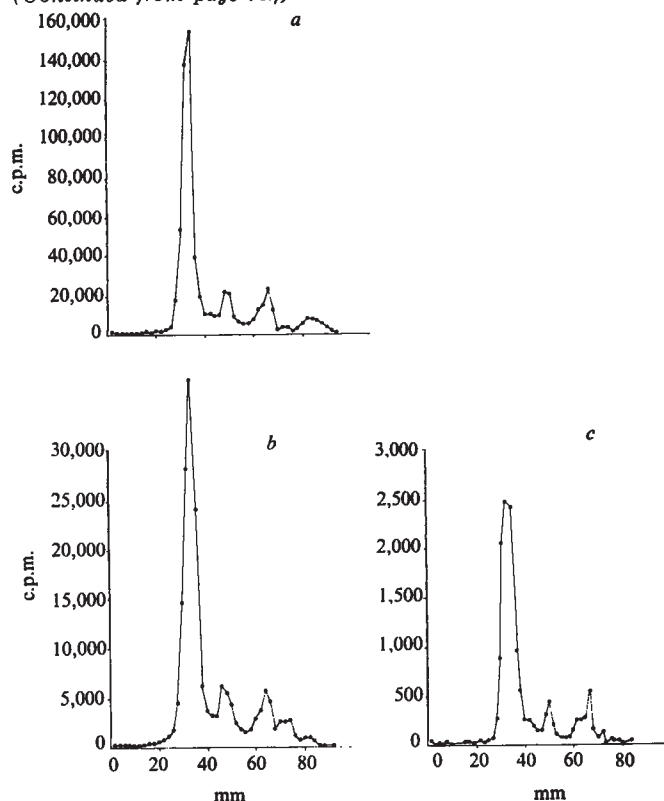


FIG. 2 Separation of iodinated rat erythrocyte polypeptides. Erythrocytes were isolated and iodinated as described in the text. Labelled cells were injected into animals and blood samples were collected at various times thereafter. Erythrocytes were washed free of serum proteins, and the plasma membranes were isolated. Membranes were then solubilised in 2–3% SDS and separated by electrophoresis on 5% polyacrylamide gels containing 0.1% SDS⁶. The distribution of radioactivity in the gels was determined by slicing the gels into 2 mm slices and then determining the radioactivity in each slice. The profiles shown are from erythrocyte membranes isolated from animals 1 h (a); 19 h (b); and 404 h (c), after injecting labelled cells.

components of larger molecular weight are degraded^{5,6} more rapidly. In the erythrocyte membrane this apparently is not the case; all components seem to have approximately the same half-life which is significantly shorter than the half-life of the whole cell.

There may be several reasons for the short half-life of membrane components observed with the iodine probe. One possible reason is that the labelled components are de-iodinated. There is a report of de-iodination activity in the erythrocyte membrane¹⁶. It is also possible that the proteolytic activity^{17,18} present in the red cell may account for the removal or loss of label due to proteolysis of the membrane component.

The most probable explanation for the short half-life observed with the iodine labelling is based on the observation that young erythrocytes are larger than old ones. The decrease in cell size is probably due to sloughing off of the membrane (see, for example, ref. 19) within the sinusoids of the spleen and liver. This process takes with it relatively small quantities of the internal components of the red cell, but probably relatively significant quantities of membranes with much smaller losses of the internal components of the red cell. Thus, by this mechanism, iodine would not be eluted in the same manner as chromium is presumed to be eluted, but would represent loss of membrane from the cell during the course of its life. Thus, membrane labelling may have advantages in the study of membrane diseases and the survival of the membrane in contrast to the interior of the cell. This method may provide information about the activity or

functional capacity of the spleen and hepatic sinusoid cells in diseases where it is possible that these organs and not the erythrocytes are defective.

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Novel human serum protein from fibroblast plasma membrane

CELL surface components play a major role in the control of normal cell interactions and in the altered growth control and antigenic properties of malignant cells¹. To characterise tissue-specific cell surface proteins we undertook an immunochemical study of the material released from cells by mild proteolytic treatment. Our studies have disclosed a fibroblast-specific cell surface and serum antigen in chicken². The amount of this antigen is greatly reduced in chicken fibroblasts transformed by Rous sarcoma virus.

These studies prompted us to search for an analogous protein in humans in light of its possible significance in human malignancies. We describe here a new cell surface protein of human fibroblasts which is also present in human serum, and seems to be the human counterpart of the chicken fibroblast surface antigen.

Antisera were raised against material prepared from the surface of human secondary fibroblasts with insoluble papain² (Enzite-EMA, Miles-Yeda). The antiserum prepared against the papain-released material from fibroblasts, gave a single precipitation line against the immunogen and similar material from four continuous fibroblasts lines: WI-38 (ref. 3), MRC-5 (ref. 4), IHM (human embryonic fibroblast) and 377HEL (human embryonic fibroblast). An antigen in-

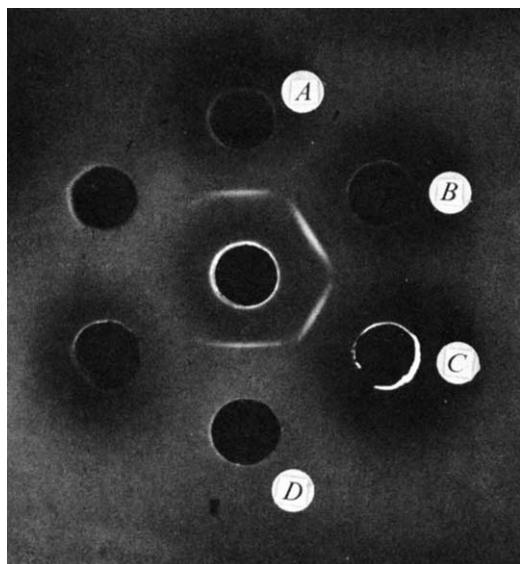


FIG. 1 Immunodiffusion in agarose. A and D, Normal human serum; B, papain digest from MRC-5 cells; C, MRC-5 cells extracted with 8 M urea and 1% Triton X-100. Centre well, rabbit antiserum prepared against papain digest from human fibroblasts.

distinguishable from the fibroblast antigen in immunodiffusion and absorption experiments was present in human serum (Fig. 1). The antigen could be solubilised from fibroblasts by papain, trypsin, or by treatment with a solution containing 8 M Urea, 1% Triton X-100, 5×10^{-5} M phenylmethylsulphonyl fluoride and 0.02% sodium azide. Incubation of the cells in buffered saline or EDTA in conditions comparable to the enzyme treatment did not yield detectable amounts of the antigen. Small quantities could, however, be detected in the maintenance medium of fibroblast cultures after 24 h incubation. The antigen was not detected in material released by papain or urea-Triton X-100 prepared from several non-fibroblast human cell lines. The cell lines included U and WISH transformed amnion lines, primary

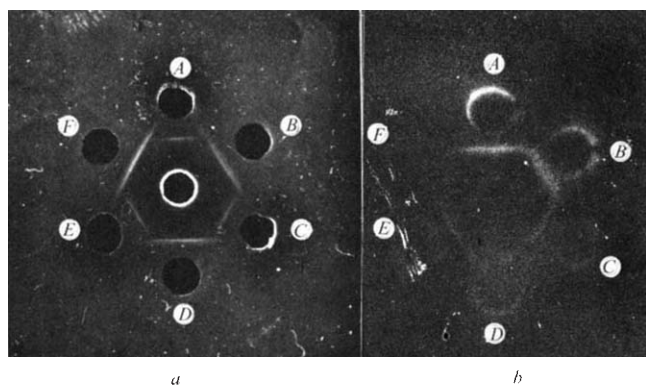


FIG. 2 Immunodiffusion in agarose (a) followed by autoradiography (b). A, C and E, Normal human serum; B, D and F, foetal extract. Centre well, rabbit antiserum prepared against papain digest from human fibroblast and anti- α -foetoprotein serum. Papain digest from H^3 -fucose labelled MRC-5 cells was added to the peripheral wells, undiluted (A and B) diluted 1:4 (C and D) and 1:16 (E and F). The highest amount of H^3 -fucose labelled digest, in well B, gives a precipitate without carrier antigen from normal human serum. Smaller concentrations in wells C and E coprecipitate with the line between normal human serum and antiserum, while the unrelated control precipitate (alpha-fetoprotein) from wells D and F remains non-radioactive.

normal amnion, HeLa cervical carcinoma, KB (nasopharyngeal carcinoma), Hep-2 (laryngeal carcinoma), P3HR-1 (Burkitt's lymphoma). Material released by papain from washed red blood cells and buffy coat leukocytes was also negative.

That the antigen is synthesised by the fibroblasts and not absorbed from serum was shown by its presence in the established human fibroblast lines grown for over 30 passages in the presence of calf serum which does not contain the human antigen. Cells grown in the presence of 35 S-methionine incorporated radioactivity to the antigen as shown by autoradiography of immunodiffusion precipitates. Similarly, radioactivity was incorporated into the antigen from 14 C-fucose (Fig. 2). Iodination (125 I) using the peroxidase method⁵ labelled the antigen as demonstrated by coprecipitation of radioactivity into a specific immunoprecipitate.

Assay of the antigen after solubilisation of cells in urea and Triton X-100 showed that treatment with trypsin (0.25%, 15 min), such as used in the subcultivation of fibroblasts, removed more than 90% of the antigen from cells without any detectable cell destruction. The antigen was fully restored within 24 h after such treatment. Absorption of the antiserum with living fibroblasts abolished its precipitating capacity in immunodiffusion. These data show that the antigen is an integral membrane glycoprotein and is exposed on the cell surface. No immunological cross reactions were demonstrable between the human and chicken² antigens. Antisera against the human material did not react with extracts from chicken cells or *vice versa*.

We have determined some properties of this protein for which we propose the name soluble fibroblast antigen (SF antigen). In sedimentation analysis we used 5–20% sucrose gradients and human IgM (19S), IgG (7.1S), albumin (4.3S), ovalbumin (3.6S) and pancreatic RNase (1.9S) as reference markers. The S values were calculated according to Martin and Ames⁶. The serum SF antigen sedimented during ultracentrifugation in the 13.5S region. In agreement with the relatively high molecular weight suggested by the S value, the serum SF antigen was precipitated by 25% saturated ammonium sulphate. On immunoelectrophoresis, the serum SF antigen gave a precipitate in the α_2 -region (Fig. 3). The presence of sialic acid in the antigen was indicated by an altered electrophoretic mobility after neuraminidase (Behringwerke) treatment of partially purified serum antigen (Fig. 4). Pronase (Calbiochem, 537088) treatment of partially purified SF antigen destroyed its antigenic activity. For inactivation by Pronase it was necessary to use slightly larger amounts than those required to abolish antigenic reactivity of human serum albumin. Human blood group A substance, used as another control, was not affected by the treatment. These data indicate that the polypeptide chain is involved in the antigenic activity.

The serum SF antigen could be demonstrated in all 60 normal human sera studied and also in smaller quantities in 2 foetal sera. We are now investigating the quantity and possible compositional deviation of the serum SF antigen in various forms of connective tissue disease and in malignancies. The absolute concentration of SF antigen in serum or cells cannot be given at present, because attempts to purify the serum SF antigen in immunoreactive form have so far been hampered by its insolubility after purification procedures. This is in line with the postulated membrane origin of the serum SF antigen, as membrane proteins tend to be hydrophobic. The antigen, however, was detectable in immunodiffusion (sensitivity about $10 \mu\text{g ml}^{-1}$)⁷, in normal human serum diluted 1:10 and in fibroblast samples containing 2.0 mg ml^{-1} of total cell protein. This result indicates that the serum concentration of SF antigen is about $100 \mu\text{g ml}^{-1}$ and it may comprise as much as 0.5% of the total cell protein, which would make it a major component of the plasma membrane.

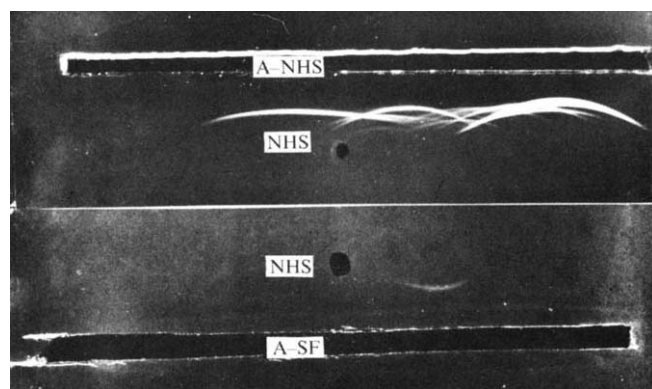


FIG. 3 Immunoelectrophoresis in agarose. Normal human serum (NHS) tested against anti-normal human serum (A-NHS) and an antiserum prepared against papain digest from fibroblasts (A-SF). Anode to the right.

The SF antigen seems to be the first well defined example of a new type of serum protein, which originates from the cell surface. Other cell surface components, also seem to exfoliate from the cell membrane and appear in circulation, albeit at minute concentrations⁸⁻¹⁰. That the SF antigen is present in serum at high concentrations may be because of the abundance of fibroblasts in the body.

Our recent data (P. Kuusela, E. R., and A. V., unpublished) indicate that the polypeptide composition of the SF antigen in serum and in fibroblasts is very similar, consisting of two major polypeptide chains with molecular weights of about 210,000 and 145,000. This is true of human as well as chicken SF antigens. This shows that the similarity of the cellular and serum antigens is not restricted to a limited antigenic determinant, but is based on a molecular similarity if not identity. This, and the fact that cultured fibroblasts shed the antigens to growth medium, strongly supports the notion that the serum antigen originates from the fibroblast surface.

As a well defined glycoprotein SF antigen may offer a unique possibility to look into the biological significance of circulating cell surface components, it has been suggested that growth regulation¹¹ and the maintenance of immunological tolerance¹² could be mediated by such molecules.

It is predictable that cell-type specific surface glycoproteins, such as SF antigen, may be important for the interactions between normal cells and perhaps function as recognition molecules¹³⁻¹⁶. Our observation that reduced amounts of the chicken SF protein², analogous to the human protein described here, are present in virus transformed cells¹⁷, is in line with this proposal. Our recent data (A. V., and E. R.,

unpublished) indicate that human fibroblasts also lose their SF antigen after transformation. This indicates that the biological role and the possible diagnostic significance of the human SF antigen and related cell surface components in human malignancies deserve further attention.

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Composition drift in the cytochrome c cistron

IN a previous letter I showed that the distribution of base frequencies for degenerate codon positions is far from random in the only completely sequenced mRNA, the coat cistron of MS2 coliphage¹. The study reported here indicates a strong evolutionary trend among non-degenerate bases in mRNA for the cytochrome c series leading to man.

Forty-three best resolved cytochrome protein sequences have been decoded to obtain theoretical mRNA base compositions, assuming equal use within the set of codons of each amino acid². The adenine content in each theoretical mRNA is correlated with the estimated palaeontological age for that type of organism (Table 1). Although fewer fish and insect sequences are available, each age is fairly well represented. Many cytochrome c molecules from plants have been sequenced but only one, ginkgo, which is believed to be the most primitive³ and thus the closest to the line leading to man, is included to avoid weighting plants too heavily in the correlation. Simply decoding every cytochrome c ever sequenced, however, while making a bulkier analysis, would not greatly affect the trends shown.

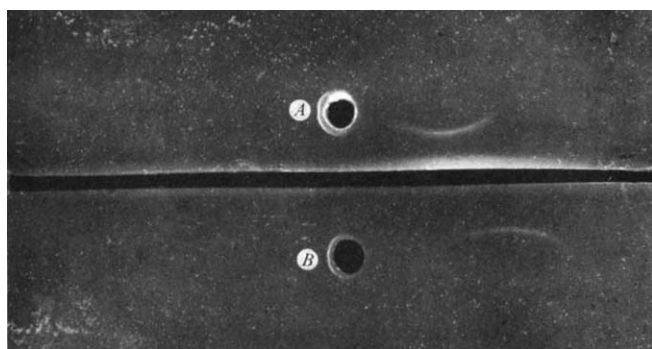


FIG. 4 Immunoelectrophoresis in agarose. Effect of neuraminidase on the electrophoretic mobility of partially purified SF antigen. A, Neuraminidase treated; B, control treated (without neuraminidase) SF antigen tested against A-SF serum.

TABLE 1 Adenine content in cytochrome mRNA

(a) Palaeontological age and adenine content of theoretical mRNA									
Bacteria, alga ^{4,11,14-17} (3 × 10 ⁹ yr)	%A	First eukaryotes ^{2,4,18-21} (1.5 × 10 ⁹ yr)	%A	Insects ⁴ (800 × 10 ⁶ yr)	%A	Reptiles, birds ^{4,23} (300 × 10 ⁶ yr)	%A	Mammals ^{4,24} (80 × 10 ⁶ yr)	%A
<i>Pseudomonas fluorescens</i> c551	28.7	<i>Crithidia oncopelti</i>	28.9	Tobacco moth	31.5	Pekin duck	35.7	Bat	35.1
<i>P. aeruginosa</i> c551	27.8	<i>Euglena gracilis</i>	31.3	Silkworm moth	31.7	Rattlesnake	35.8	Dog	35.8
<i>P. stutzeri</i> c551	27.2	Ginkgo	31.6	Screw-worm fly	31.7	Chicken, turkey	36.0	Whale	36.0
<i>P. mendocina</i> c551	25.7	<i>Candida krusei</i>	30.0	Fruit fly	31.8	King penguin	36.3	Rabbit	36.1
<i>Monochrysis lutheri</i> c553	27.8	<i>Ustilago sphaerogena</i>	32.5	Emu		Emu	36.4	Pig, cow, sheep	36.4
<i>Desulfovibrio vulgaris</i> c553	31.0	<i>Neurospora crassa</i>	32.6	Fish ^{4,22} (400 × 10 ⁶ yr)		Snapping turtle	36.7	Horse, donkey	37.7
<i>D. vulgaris</i> c3	32.9	<i>Debaromyces klockeri</i>	33.0	Carp	32.8				
<i>D. gigas</i> c3	31.8	Baker's yeast	34.2	Tuna	34.0	Bullfrog (350 × 10 ⁶ yr)	35.1	Kangaroo (100 × 10 ⁶ yr)	36.7
<i>D. desulfuricans</i> c3	29.8			Bonito	34.1				
<i>Rhodospirillum rubrum</i> c2	33.4			Puget Sound dogfish	34.7			Man, monkeys (present)	36.6
<i>Chloropseudomonas ethylica</i> c7	34.7			Pacific lamprey (450 × 10 ⁶ yr)	34.8				
(b) Correlation between age and %A in theoretical mRNA									
No. organisms	Span: from man to	-r*	-b*	a*					
24	Insects	0.89	6.5	37.1					
32	First eukaryotes	0.82	3.3	36.2					
43	All bacteria + alga	0.77	2.0	35.6					
36	<i>Pseudomonas</i> c551 only	0.91	3.0	36.1					

* r is linear regression correlation coefficient, b is slope (%A per 10⁹ yr), a is intercept at time zero (present).

I have tried to take a least controversial figure for each evolutionary age. Ages of bacteria and algae are generally estimated at around 3×10^9 yr^{3,4}, first eukaryotes at 1.2×10^9 to 1.8×10^9 yr^{5,6} and insects at 600–1,000 Myr^{4,7,8}; agreement is better on dates for more recent organisms (from lamprey onwards). The conclusions of this paper would not change if some of the ages in Table 1 are wrong by 25%–30%.

The adenine content in cytochrome *c* theoretical mRNA has increased by about 5% (absolute) since the appearance of eukaryotes. This is an enormous value in a cistron which already contained a considerable amount of A (29%–33%) at that time and which evolves slowly. Just when the trend started is uncertain, however, because the ancestry of cytochrome *c* in bacteria and algae is not clear^{4,6,9-11,12}. The trend may be accelerating, as a comparison of the magnitudes of the three slopes suggests (human–insect > human–first eukaryotes > human–bacteria and alga, see Table 1b). But perhaps insect divergence goes back almost to the appearance of eukaryotes.

A bacterial or algal ancestor cistron containing 35%–37% A is unlikely because of the lower percentage of A for intermediate organisms (protozoa, insects and fish) accepted as having cytochrome *c* homologous with that of man. Also, all fifteen theoretical mRNAs from bullfrog onwards, on the evolutionary scale, have at least 35% A whereas none of the eleven bacterial and algal ones have as much as 35%. In fact, judging composition trends, *Pseudomonas* c551 is a more likely candidate for the ancestor of mammalian cytochrome *c* than is *Rh. rubrum* c2. Mammalian cytochrome *c* has 72% (relative) more Lys and 38% more of the combined five amino acids besides Lys with a high percentage of A in their codons (Asn, Gln, Glu, Ile, Thr) than has *Pseudomonas* c551. Lysine codons have an average A content of 83.3% whereas codons from the other five amino acids average 50% A. As a generalisation, evolutionary change of each residue in cytochrome *c* is proportional to the adenine content of its codons. This cannot be explained by increasing polarity because two (Ile, Thr) of the five amino acids have low polarities. Furthermore, highly polar Asp (whose codons have an average of 33.3% A) has decreased in frequency while less polar Asn¹³ (whose codons have an average of 66.7% A) has increased during this interval. The evolu-

tionary progression of the six amino acids with codons of high A content is summarised in Table 2.

Neither a classical selectionist nor a neutralist interpretation of these findings seems possible. Unless a bias in the choice of organisms to sequence somehow exists, however, there is no escaping the fact of a general, progressive increase of all codons of high A content in the cytochrome *c* line. There are two conceivable models for the trend. In the first, cistron evolution proceeds by a general increase of codons containing a predominance of adenine while no special control is exerted on degenerate bases. Alternatively, the observed increase in non-degenerate A is compensated by appropriate choices among degenerate bases to contain the drift in total cistron composition⁴.

mRNA sequences will help to decide between these models. The actual mRNA in cytochrome *c* will have had to gain considerably in A content unless numerous silent mutations (ones not affecting amino acids) have occurred. This is readily seen by aligning, for example, horse and silkworm moth cytochrome *c* sequences. Ignoring non-homologous ends (last horse and first four moth residues) and noting possible base changes in codons of exchanging amino acids, the adenine content of horse mRNA can be lowered at most by 1% by avoiding A as a degenerate base in replacing codons, whereas the theoretical adenine difference between the two species is 5%.

Other proteins show trends in the choice of non-degenerate

TABLE 2 Evolutionary progression of amino acids with codons of high adenine content

No. of sequences	Type	Average in cytochrome	
		% Lys	% NQEIT*
11	All bacteria and alga ^{4,11,14-17}	13.0	19.4
(1)	<i>Rhodospirillum rubrum</i> c2 ⁴	15.2	24.0
(4)	<i>Pseudomonas</i> c551 ^{4,11}	10.1	21.1
8	First eukaryotes ^{2,4,18-21}	12.9	25.5
4	Insects ⁴	13.6	24.3
5	Fish + lamprey ^{4,22}	16.0	26.0
7	Reptiles, bird + frog ^{4,23}	17.3	28.0
8	Mammals + kangaroo ^{4,24}	17.4	29.2

* Asn + Gln + Glu + Ile + Thr.

codon bases (for example, the theoretical mRNA in haemoglobin α chain has increased sharply in C content during its evolutionary life) but fewer sequences or confirmed compositions are available for each series.

It seems, therefore at least for composition trends, that gene evolution is not following a random course. This seems a genuine case of long-term oriented molecular evolution. Whether or not archaic morphology means archaic genes, the correlation in Table 1 remains to be explained. The question is how independent are genetic and phenotypic evolution (further work in preparation).

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Fusion of fungal protoplasts

RESEARCH on fusion of cells of higher eukaryotes is in an advanced stage and excellent reviews have been published concerning fusion of both animal cells¹⁻⁴ and plant protoplasts⁵⁻⁷. On the other hand, no information is available regarding the results of fusion of fungal protoplasts, in spite of its possibilities for basic and applied research. Here we present evidence that fungal protoplast fusion and heterokaryon formation can be achieved. In our experiments no stimulants of protoplast fusion were employed. In fact, a fungus was used in which we have never observed anastomosis and heterokaryosis or sexual processes.

Nutritionally deficient stable mutants were produced from *Geotrichum candidum* SzMC 0055 with N-methyl-N'-nitro-N-nitrosoguanidine (50 μ g ml⁻¹, 60 min, 10⁴ arthrospores ml⁻¹). Auxotrophic mutants requiring adenine (*ad*), lysine (*lys*), isoleucine, and methionine were used. Nutritional complementation by protoplast fusion could be achieved with any pair of these auxotrophic mutants, but results reported here refer to the *ad* and *lys* mutants.

Protoplasts were formed from rapidly growing hyphae (100 mg fresh weight ml⁻¹) after 2-mercaptoethanol treatment⁸ by using lyophilised snail digestive juice (1.5%), with 0.6 M KCl or 0.8 M mannitol or glucose as osmotic stabiliser at pH 6.5. Almost complete protoplast formation was attained within 2 h. After enzyme removal, protoplasts (10⁸ ml⁻¹) from the two mutants were mixed in a ratio of 1:1 and 10 ml portions in the osmotic stabilisers at pH 5.5 and 7.0 were sedimented in 1 cm diameter centrifuge tubes at 2000g. The sedimented protoplasts were kept at 4 or 20° C for 12 h, resuspended, plated on osmotically stabilised minimal medium containing 2% agar and covered with a thin layer of the same agar medium by spraying. The hot agar solution (approximately 80° C) was sprayed in sterile conditions with the aid of an atomiser from the distance of about 50 cm. The temperature of the agar droplets was about 30° C at the time of reaching the surface of the inoculated plates. Slightly better results could be obtained if the surface of the plates were allowed to dry before spraying. The thickness of the covering layer was about 0.2 mm. By using the spraying method, almost 100% protoplast regeneration could be achieved with the prototrophic *Geotrichum* strain, or with auxotrophic mutants in supplemented media. Plates were incubated at 30° C and checked for nutritional complementation.

With mannitol or glucose as stabiliser, complementation occurred with low but constant frequency. At 20° C the average frequency of complementation was 1.6×10^{-6} per protoplast pair at pH 5.5, and 6×10^{-7} at pH 7. At 4° C the proportion of complemented cells was even lower. Complementation did not result with KCl as osmotic stabiliser.

No colonies appeared from separately treated protoplasts of the auxotrophic mutants; nor if arthrospores or growing hyphae of the two mutants were mixed, kept together, and plated as for protoplasts; nor if mixed but not sedimented protoplasts were plated; nor if protoplasts of one mutant were mixed with burst protoplasts from the other mutant.

Colonies derived from complemented cells differ char-

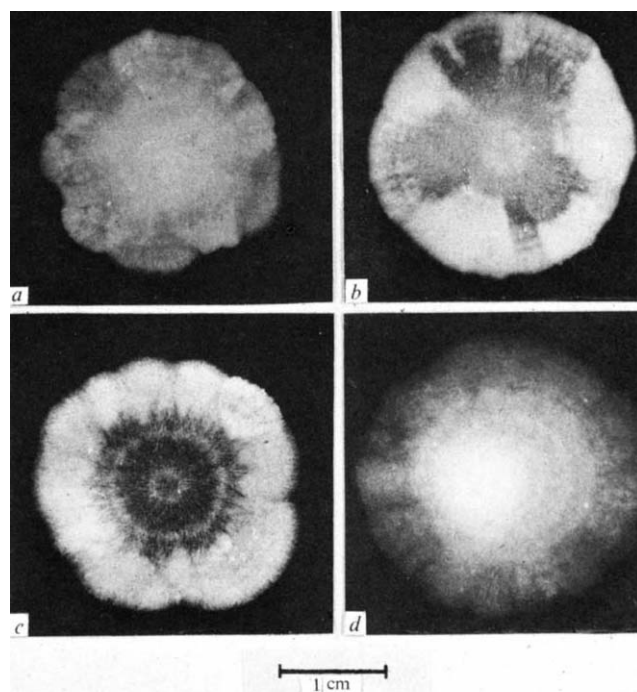


FIG. 1 Macrocolonies of *Geotrichum candidum*. a, Prototrophic strain on minimal medium; b, Heterokaryon (*ad*+*lys*) on minimal medium; c, *ad* mutant on minimal medium supplemented with 50 μ g ml⁻¹ adenine; d, *lys* mutant on minimal medium supplemented with 50 μ g ml⁻¹ lysine.

acteristically in colour and morphology from the colonies of the original prototrophic strain and those of the parent auxotrophic mutants. The prototrophic strain and the *lys* mutant are white, the *ad* mutant is red; colonies of heterokaryons are different shades of pink. The morphological differences are illustrated in Fig. 1.

Serial transfer of the complemented hyphae could be performed indefinitely using minimal medium. Complementation, however, was temporary. Complemented hyphae transplanted using media rich in adenine and lysine gave rise mainly to auxotrophic arthrospores. In agar cultures, the proportion of segregation into *ad* and *lys* mutants differed from colony to colony. Between the segregants, the *lys/ad* quotient proved always higher than 1, with the highest value of 6.5 and the lowest of 1.4. The average ratio in colonies 2 d old was 6:3:2, *lys*, *ad* and complemented arthrospores respectively. This ratio changed with time, the growing colonies bearing proportionally fewer and fewer complemented arthrospores.

Colonies developed from complemented arthrospores again bore nutritionally deficient arthrospores. Permanently complemented cells have not been observed up to the present.

From complemented hyphae grown on minimal medium, mostly complemented arthrospores were obtained, although arthrospore formation was rare, especially within the liquid. A considerable number of auxotrophic colonies, however, could be regenerated from protoplasts produced from complemented hyphae after repeated protoplast formation.

Nutritionally deficient stable mutants of *Geotrichum candidum* offer many technical advantages in studies of the conditions required for a more efficient means of fungal protoplast fusion. Further studies with *Geotrichum candidum* and other species to increase the frequency of successful fusion are in progress.

It may be mentioned that morphological alterations are not confined to *Geotrichum*; after protoplast fusion of nutritionally deficient *Aspergillus nidulans* mutants, well-defined differences can also be observed between colonies of the wild-type strain and those of the heterokaryons.

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examples of two types of abnormal plants induced by two different strokes of the sledge-hammer, and Fig. 1a, Fig. 2a, Fig. 1b, and Fig. 2b, show corresponding stages of the wild type and two developmental mutants.

Normally the wild type starts its development by forming a filament consisting of a row of cells. When the filament is about 16 cells long, the division planes become orientated through the longitudinal axis and the germling is, essentially, transformed into a tube closed at both ends. The proximal part differentiates into a holdfast with large stem cells and elongated rhizoid cells, while the distal part flattens and expands as a sheet consisting of a double layer of polygonal cells.

A number of mutants have been isolated, which instead of the three morphologically distinct cell types of wild type contain only one or two. Some of these mutants pass a filamentous stage¹ but others do not and develop as hollow spheres² or as aggregates of cells³. At least some of these mutations seem to be in genes active during early stages of development^{4,5}.

The direction of the first division plane of wild type can be set by applying unidirectional light or an electrical field for a certain period about 24 h before the first division⁶. The division plane is then perpendicular to the vectorial stimulus and to the surface on which the cells are growing. The following planes are always parallel to the first and cannot be influenced by light or an electrical field. Filamentous growth, however, is not dependent on a vectorial stimulus of any duration, because normal development occurs when zygotes are grown in agitated suspensions. Centrifugal forces up to 280,000g or ultrasonic treatment given before the first division, do not affect development. Morphogenesis is also normal when the amount of light is reduced and growth is extremely slow.

Until now, the only treatment we have found which affects morphogenesis is exposure of the zygotes to mechanical shocks. The idea came from the work of Kamiya and Kuroda⁷, whose 'hammer experiment' succeeded in releasing *Nitella* chloroplasts from their cortical positions.

Zygotes were prepared⁸ and allowed to settle overnight on cover slides 18 × 18 mm. Thereafter, they were exposed to routine culture conditions⁴ with a light: dark cycle (0400h–1700h, light; 1700h–0400h, dark) starting about 9 a.m. The following day the glass, with hundreds or thousands of zygotes, was placed on the honed surface of a sledge-hammer (Fig. 3) and kept there by an O-ring pressed against the glass by a lid fastened with three screws. An anvil was struck with the other end of the hammer. The zygotes were then cultivated further and a clearcut effect could at the earliest be observed when the zygotes started to divide

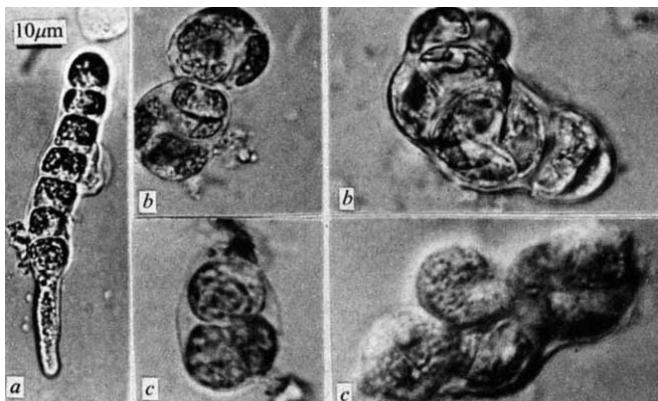


FIG. 1 a, Wild-type germling; b, two developmental stages of the mutant lumpy; c, two developmental stages of a wild-type zygote exposed to mechanical shocks at an age of 1d (2 d before the first cell division in the controls).

Mechanical shocks induce phenocopies of developmental mutants

IN the multicellular green alga *Ulva mutabilis* Føyn, the early division pattern and cellular differentiation can be influenced by exposing the zygotes to mechanical shocks created by a sledge-hammer. The treated plants mimic certain mutants with an abnormal division pattern and/or with less cellular differentiation than wild type. Figure 1c, and Fig. 2c, show

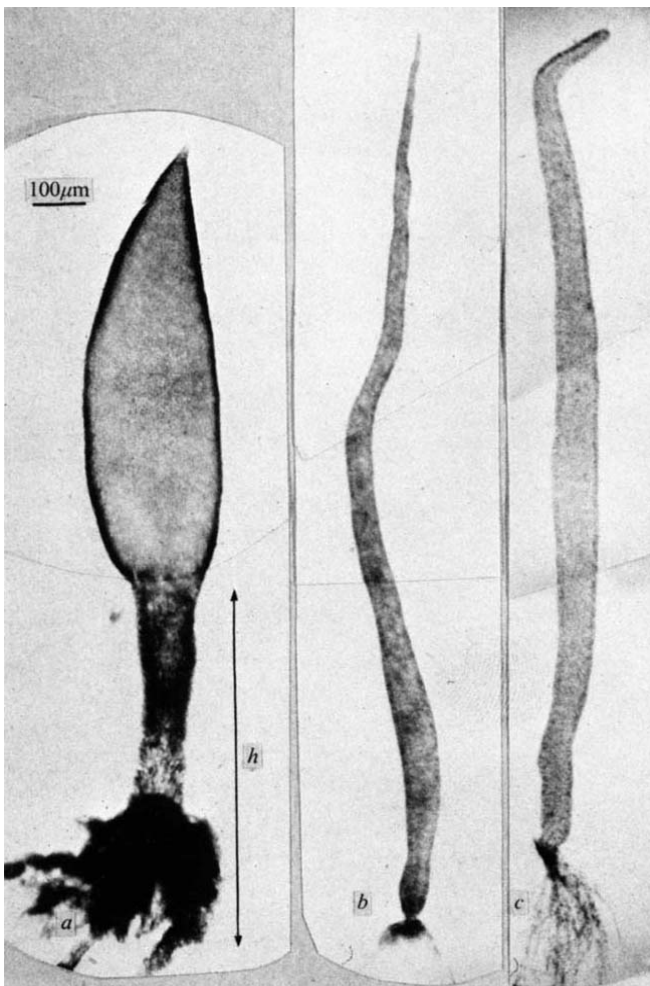


FIG. 2. *a*, Wild-type plant about 4 weeks old (*h*, holdfast); *b*, mutant 4b about 4 weeks old; *c*, wild-type plant about 4 weeks old. The plant was exposed to a mechanical shock as a 1-d-old zygote.

48–72 h later. The possibility that the effect of the stroke is created by a sudden increase in hydrostatic pressure within the O-ring is excluded, because plants outside the ring also react.

Our first experiment showed that all zygotes on a glass developed in the same way. Of 11 successive strokes, four gave normal plants, two gave plants which did not form a proper filament and showed delayed differentiation, and five gave plants which passed a filamentous stage, but did not differentiate a proper holdfast. Numerous other experiments have given similar results, and the two types of abnormal plants have been recorded repeatedly.

The first kind of abnormal development is illustrated in Fig. 1c. The division planes are arbitrarily arranged and the plants develop as cellular aggregates similar to those of the mutant *lumpy* (Fig. 1b). The walls between the cells appear curved instead of straight. Even before division, there is the impression that something is wrong with wall architecture and at high magnification the outline of the walls seems thicker and more irregular than normal. The generation time of the cells is longer than usual and differentiation is delayed for weeks if not months. The change induced by the stroke should therefore be considered heritable through many cell generations. Finally some cells regain normality and wild type plants sprout from the aggregate.

The second kind of effect is illustrated in Fig. 2c. The plants develop as filaments and are transformed into tubes. The proximal differentiation of the large stem cells and the flattening of the distal part does not take place. They re-

produce much earlier than wild type. The effect of the stroke can already be seen on the filaments. In wild type, the width of the cells decreases from base to apex but in the treated plants the cells are more uniform. The development of secondary rhizoids is also delayed compared to the controls, but a fair amount of rhizoid cells will develop and fasten the plant to the substratum. The abnormal plants mimic a certain type of developmental mutants (Fig. 2b) not previously described.

In one experiment, zygotes 1, 2, 3 and 4 d old were treated. Ten blows were struck each day, and of them five, four, three and five strokes respectively had an effect on development. There is therefore no indication of a period before the first division, when the zygotes are especially vulnerable. When the head of the sledge-hammer was dropped through an iron pipe onto the anvil from heights of 6, 12 and 18 m, three of eight, nine of eleven and four of eleven experiments gave abnormal development. The experiments were repeated with a steel cylinder, made to accommodate the zygotes in the same manner as the sledge hammer, but numerous trials from the same and even greater heights, gave no effects at all. The cylinder was a little shorter than the sledge hammer, had no hole for a shaft, but was given the same hardness as the hammer. No effects were obtained when the cylinder was fastened to a shaft by a ring-shaped holder and used as a hammer.

Our experiments show clearly that the wild-type zygotes of *Ulva mutabilis* can be induced to mimic the development of certain mutants. It is important to note that either the plants do not react at all to the treatment, or every single plant of the exposed population reacts by following a particular abnormal developmental path. It is also of significance that effects were obtained with a particular sledge hammer, and that the results could not be repeated by using one of another form. These facts raise the question if the different effects are due to different shock waves, created because one stroke is not exactly like another.

It is common opinion that the early development depends on the cytoarchitecture of the cell, especially the cortex, and it is therefore possible that the treatment distorts the cytoarchitecture of the zygotes in an irreversible manner. There is the most interesting possibility of changing the

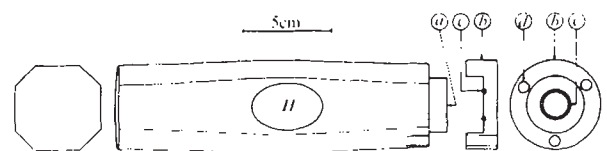


FIG. 3 The sledge-hammer with which the effects were obtained. *a*, The honed surface on which the cover-slide with the zygotes was placed; *b*, the lid with O-ring; *c*, and three screw-holes; *d*, to press the cover slide against the surface. *H*, Hole for the wooden shaft. The hardness of the steel was 68 Rockwell. The sledge hammer was made for the Municipal Tramcar Company in Oslo about 50 yr ago and specimens are no longer available.

cytoarchitecture in defined directions. Our experimental arrangements are primitive but we hope this note will bring us in contact with people who can suggest more appropriate methods, which enable measurements and calculation of the velocity, amplitude and shape of the shock wave.

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Differential survival of skin and heart allografts in radiation chimaeras provides further evidence for Sk histocompatibility antigen

CERTAIN inbred mice, lethally irradiated and inoculated with bone marrow and spleen cells from F1 hybrids, reject donor-strain skin grafts although they are permanent haemopoietic chimaeras¹⁻³. After rejection their serum is specifically cytotoxic *in vitro* for donor-strain epidermal cells but not lymphoid cells⁴, and their leukocytes are stimulated specifically in mixed cultures by donor-strain epidermal cells but not leukocytes⁵. Thus epidermal cells contain distinctive surface alloantigens (skin-specific or 'Sk' alloantigens) which have been suggested to function *in vivo* as histocompatibility antigens. If this is correct, the rejection of donor-strain skin grafts by persistent chimaeras could be explained as a loss of 'self-tolerance' to donor-strain Sk antigens by grafted haemopoietic cells as they proliferate in allogeneic recipients in the absence of these antigens^{1,2}. This interpretation is strengthened because donor-strain skin grafts are not rejected if radiation chimaeras receive donor-strain Sk antigen in the form of epidermal cell suspensions at the time of marrow restoration and thereafter⁶.

Working with inbred rat strain combinations which differ considerably in the survival of skin and organ allografts, we were attracted to the proposed Sk histocompatibility system because of its potential importance as a model of tissue-specific allograft reactions. For example, by analogy with the rejection of skin allografts in the face of persistent haemopoietic chimaerism, the rejection of skin allografts in situations where heart transplants survive indefinitely might result from alloantigens present in skin but absent from heart tissue. Alternately, the differential survival of skin and organ allografts might depend on non-specific differences in their mode of transplantation; natural vascularization and prolonged ischaemia compared with prompt surgical anastomosis, quantitative differences in graft antigenicity, different routes of sensitization and so on.

Previously we attempted to minimize non-specific differences between skin and heart transplants by grafting neonatal heart fragments subcutaneously⁷. Such allografts were rejected acutely like skin allografts, even though surgically

anastomosed heart organ allografts from the same donor strain survived indefinitely^{8,9}. Although this gave no evidence for Sk histocompatibility antigens in rats, they were not ruled out. Moreover, reports that surgically anastomosed skin allografts were rejected acutely in a rat strain combination where kidney allografts survive indefinitely^{10,11} suggested the presence of Sk antigens.

The ability (of J. S. L.) to anastomose organ transplants in mice has facilitated comparison of the survival of surgically anastomosed with naturally vascularized allografts in mouse strain combinations where Sk histocompatibility antigens are presumably operative. As in our experiments with rats, our rationale was to control the non-specific factors in grafting different tissues to assess the importance of tissue-specific factors. We now report the first results of our experiments with heart allografts in mouse radiation chimaeras that reject donor-strain skin allografts while remaining permanent haemopoietic chimaeras.

We produced Sk-reactive radiation chimaeras as described by Boyse *et al.*¹, by exposing C57Bl/6 (B6) mice to 750r whole-body X-irradiation and then inoculating them intravenously with about 50×10^6 (B6 $\times$ A)F1 hybrid spleen cells. As controls for the effects of irradiation we inoculated other similarly irradiated B6 mice with B6 spleen cells. After 11-12 weeks we sent citrated blood samples from the presumed B6/B6 $\times$ A chimaeras to Dr E. A. Boyse's laboratory for H-2 typing of erythrocytes¹. One to three weeks later (that is, 12-15 weeks after irradiation and cell restoration) we challenged those mice with clear evidence of B6/B6 $\times$ A chimaerism and the B6/B6 controls with various types of strain A test grafts. All the skin grafts were transplanted orthotopically to the thorax and evaluated in terms of grossly observable epithelial survival as described by Billingham¹². Since it is necessary to use perinatal donors to obtain heart fragments that acquire assayable pulsatile activity⁷, in one series skin grafts were taken from the newborn mice that provided the heart fragments in order to control for any possible reduced immunogenicity of neonatal tissue. Moreover, 2-mm diameter "mini"-sized grafts were used with the intention of approximating the tissue antigen dosage of the heart fragments. Adult heart organs were transplanted heterotopically to the abdomen as described by Corry *et al.*¹³; newborn heart fragments were transplanted subcutaneously to the ear as described by Fulmer *et al.*⁷. Both types of heart grafts were monitored electrocardiographically as well as grossly and rejection was defined as permanent cessation of electrical activity.

Table 1 shows B6/B6 controls rejected both types of skin and heart test grafts acutely. Indeed, these survival times are similar to those for comparable test grafts in intact non-irradiated B6 recipients, indicating that there were no apparent residual effects of radiation. In contrast, there was a striking difference in the fate of the skin and heart test grafts in the chimaeras. The chronic rejection of the skin grafts verifies the observation of Boyse *et al.*^{1,2} No heart test graft

TABLE 1 Survival of skin and heart test allografts in radiation chimaeras and controls

Type of strain A test graft	Number	B6/B6 controls MST $\pm$ s.d.*	Test graft survival (d) in:			
			Range	Number	B6/B6 $\times$ A chimaeras MST $\pm$ s.d.	Range
Adult donors						
skin	7	8.7 $\pm$ 1.1	8-10	10	20.2 $\pm$ 1.4	14-36
heart organ	8	9.0 $\pm$ 1.4	7-12	5	(66+ [†] , 70+ [†] , 146 [†] , 162 [†] , 175 [†])	
Newborn (0-48 h-old) donors						
'mini' skin§	6	7.3 $\pm$ 1.3	8-12	11	27.0 $\pm$ 1.4	18-52
heart fragment	7	13.5 $\pm$ 1.1	12-16	10	(All 175 [†])	

* Median survival time $\pm$ s.d. as computed by Litchfield's nomograph method¹⁶.

+ Died with surviving test graft.

† Alive with surviving test graft as of November 30, 1973.

§ 2-mm diameter as opposed to the 15-mm diameter adult skin grafts.

recipient has rejected its test graft so far, although two died with surviving grafts. Three months after skin grafting, and well after rejection of the last test graft, we again sent blood samples to Dr Boyse for tissue typing. In every case but one, chimaerism had persisted in spite of the donor-strain skin allograft rejection. Moreover, 2 months after heart grafting, all recipients, when challenged, rejected strain A skin test grafts without discernable effect on preexisting heart grafts which, as just indicated, are still surviving.

We draw two conclusions from the differential survival of skin and heart allografts in our chimaeras. First, in the strain combination tested there is no evidence that mouse heart tissue possesses distinctive histocompatibility alloantigens analogous to Sk. It would, however, be unsafe to conclude that such antigens are not present in heart cells. Even in the case of Sk antigens a serological anti-SK response has been demonstrated in the absence of graft rejection⁴, as is also the case in the H-Y¹⁴ and Θ ¹⁵ incompatibility systems. Second, the case for Sk functioning as histocompatibility antigen is strengthened, particularly by the differential survival of the newborn skin and heart fragment allografts. Here, where a deliberate attempt was made to graft skin and heart tissue under comparable non-specific conditions (a strategy for revealing the possible contribution of tissue-specific factors), the differential survival observed with the conventional skin and heart organ allografts from the adult donors was maintained.

Our results provide further evidence the Sk antigen can function as histocompatibility antigen. However, they do not rule out an important role for non-specific factors^{8,9} in acute skin allograft rejection because the roles of tissue-specific and non-specific factors are not mutually exclusive. The only definitive way of determining the relative *in vivo* importance of anti-Sk immunity may be through the creation of a congenic resistant line of mice differing from an inbred strain only at the presumed Sk locus.

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Pharmacological evidence related to the existence of two sodium channel gating mechanisms

IN 1952, Hodgkin and Huxley¹ proposed the now classical differential equation that has become the accepted mathematical model for describing action potentials. In this equation there are three probability factors, m , n and h , which depend on transmembrane potential and time. Conceptually these probability factors are often considered to represent three field-dependent molecular gating mechanisms that mediate ionic flow through two transmembrane channels. The m and h gates mediate sodium currents and the n gate controls potassium flow. Electrical evidence has been presented for a molecular rearrangement associated with sodium activation (for example, m gate opening)². This evidence is based on the detection of transient electrical currents in the axon membrane which are hypothesised to represent rearrangement of molecular dipoles.

Pharmacological evidence for these gating mechanisms is scanty. Purified conylactis toxin³ has been reported to interfere with sodium inactivation⁴, but only in invertebrates (personal communication from B. I. Shapiro), thus restricting its usefulness and generality. Tetrodotoxin and saxitoxin are believed to bind to a common site on the sodium channel^{5,6}, but so as to decrease maximum sodium permeability, $\bar{g}_{Na}$, rather than affecting any of the probability factor equations. The difficulty of synthesising active derivatives of these complex alkaloids limits their usefulness as biological probes of structure-activity.

We have been investigating neurotoxins that act specifically on action potentials in vertebrates and may therefore be useful as probes of the nervous conduction mechanism in higher animals⁷. The toxins are components of the venom of the scorpion *Heterometrus gravimanus*. Two active fractions have been isolated one of which seems to delay sodium inactivation while the other exerts an effect which may be consistent with an abnormally persistent sodium activation.

Lyophilised venom of *Heterometrus gravimanus* (Sigma) was fractionated on a column of Whatman 23 CM-cellulose as shown in Fig. 1. Seven peaks led absorption above back-

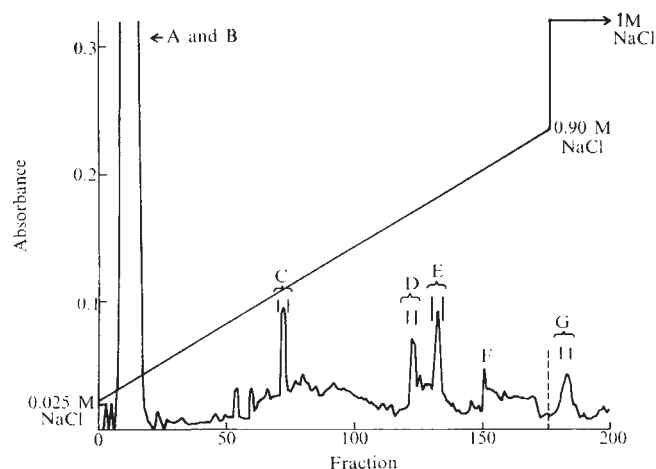


Fig. 1 Chromatogram of *Heterometrus gravimanus* venom on carboxymethyl-cellulose (Whatman 23). Absorbance was measured at 280 nm.

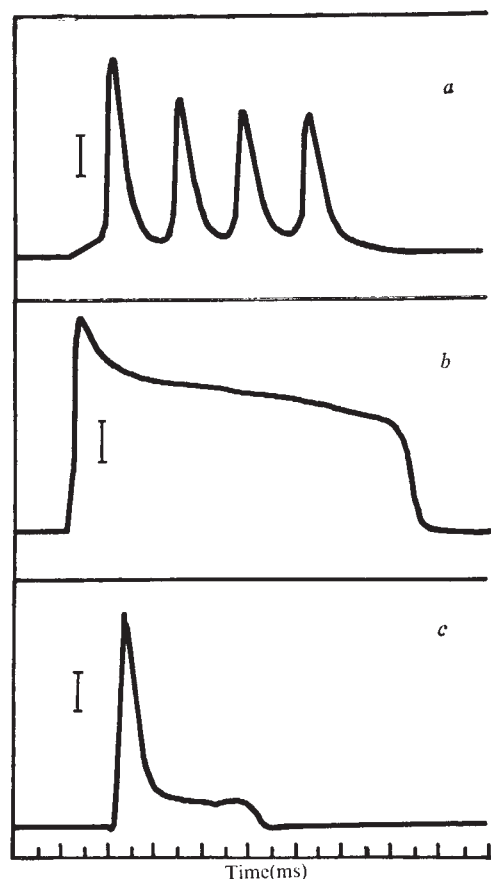


FIG. 2 Action potentials recorded at the node of Ranvier from sciatic nerve fibres of *Xenopus laevis* after application of venom or venom fractions. Procaine (1%) was used to block biphasic potentials. The vertical markers represent a scale of approximately 20 mV. *a*, Crude venom ($20 \mu\text{g ml}^{-1}$); *b*, fraction D from chromatographed venom ($1 \mu\text{g ml}^{-1}$); *c*, fraction C from chromatographed venom ($1 \mu\text{g ml}^{-1}$).

ground at 280 nm, and all peaks gave positive tests for protein by the method of Lowry *et al.*⁸ Table 1 gives this data in more detail. It is interesting that fractions C and D, the only two that affect action potentials, each represent less than 1% of the venom by weight.

Action potential responses were obtained by a technique described earlier^{9,10}, in which single medullated nerve fibres from the sciatic nerve of *Xenopus laevis* were recorded using a single air gap technique. In some experiments, 0.11 M KCl was used to block the biphasic action potential on one side of the air gap and in other experiments procaine was used. Continuous flow of fluid over active nodes in the opposite pool adjacent to the air gap was maintained relatively constant by means of gravity flow from a Mariotte flask.

When crude venom was applied to a node for several minutes in a dose of $20 \mu\text{g ml}^{-1}$, repetitive action potential spikes resulted (Fig. 2*a*). There was no long delay in the inactivation process as had been reported for the venom of *Leiurus quinquestriatus*¹¹. The effect was irreversible. However, when the venom was first dialysed against Ringer solution for 4 h and then applied to the nerve fibre, no repetitive spikes were seen, but there was a large and irreversible delay in sodium inactivation when venom was applied in a dose of $50 \mu\text{g ml}^{-1}$ (similar to Fig. 2*b*). Twenty to thirty minutes after application of venom, the delay was several hundred milliseconds. The difference between the results of experiments conducted before and after dialysis suggested that there was more than one venom component, each acting by a different mechanism. At least one of these components would be either readily dialysable or absorbed

by the membrane. Addition of 10mM tetraethylammonium chloride (TEA) to the perfusion medium failed to stop either the repetitive spikes or the sodium inactivation delay, giving further evidence that the sodium channel was the target of the venom. TEA selectively blocks potassium conductance increases¹².

Subsequent tests of fractionated venom components gave the following results. First, only fractions C and D affected the action potential. Fig. 2*b* shows an action potential from a nerve fibre poisoned by fraction D. There is an irreversible delay in sodium inactivation at concentration of $1 \mu\text{g ml}^{-1}$, similar to that observed in the crude venom of *Leiurus quinquestriatus*¹¹. With fraction C (Fig. 2*c*), however, the time course of voltage increases and decreases during the action potential was relatively unaffected until the membrane potential returned to about 20 mV above the resting level. Recovery was then characterized by a persistent plateau lasting many milliseconds. Since the initial time course of sodium inactivation seemed to be essentially unchanged, we concluded that persistent sodium activation may have been involved. This conclusion is reinforced by observations in which the crude venom of *Centroides sculpturatus* was found to prolong sodium activation¹³. Increasing the dosage or prolonging the exposure only increased the length of the trailing plateau and did not affect its potential level. TEA (10 mM) failed to abolish this plateau, although a slower spike decay was apparent, as would be expected from an action potential without the delay potassium permeability change. This effect was also irreversible.

At this stage, we have no explanation for the repetitive spikes produced by crude venom, although it may be the result of combined interactions of two or more venom components with the nerve membrane.

Positive results in the Lowry test suggested that the active components of the venom were protein. Preliminary studies on sodium dodecyl sulphate gel electrophoresis indicated molecular weights of less than 10,000, and the high NaCl molarity required to elute these fractions from the CM-cellulose indicated a positively charged protein. These properties are reminiscent of those possessed by the cholinergic neurotoxins of Elapid snake venoms. (Detailed biochemical studies will be reported later.)

TABLE 1 Protein content of each peak estimated by Lowry's method

Fraction	Absorbance (750 nm)	Est. conc. ($\mu\text{g/ml}^{-1}$)	% of Venom (weight)
A	>0.500	>140	>7.0
B	0.329	90	3.6
C	0.115	33	0.66
D	0.170	48	0.96
E	0.152	43	1.29
F	0.150	43	0.43
G	0.249	71	2.85

The instrument for measuring absorbance was calibrated with bovine serum albumen as standard.

Our results may be significant for two reasons. First, they offer possible pharmacological evidence for two molecular gating mechanisms that may be a biochemical correlate of the *m* and *h* probability factors originally derived by Hodgkin and Huxley¹. Second, and perhaps more important, these neurotoxins have potential application as previously unavailable biochemical probes of the sodium channel. A parallel situation existed with the cholinergic neurotoxins of the Elapid snake venoms in that they allowed the first widely acceptable isolations and partial biochemical characterisations of the acetylcholine receptor.

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Absorbance changes induced by blue light in *Phycomyces blakesleeanus* and *Dictyostelium discoideum*

BLUE and near ultraviolet light controls several metabolic and physiological processes in organisms ranging through bacteria¹, fungi² and algae^{3,4} to vascular plants⁵ and animals^{6,7}. The similarity of the action spectra of these various light-induced responses (all have action maxima at about 470 nm) suggests a common photoreceptor pigment system which controls these different cellular processes. We have looked for light-induced absorbance changes that might be related to such a pigment system.

Our initial work involved *Phycomyces blakesleeanus* because extensive investigations had been made of the blue-photoreceptor pigment in this organism². (This pigment resides both in the sporangiophores where it controls the phototropic response² and in the mycelium where it controls sporangiophore initiation⁸.) Mycelial mats were used rather than sporangiophores because of the greater ease of collection and preparation, and because the large vacuoles in the sporangiophores made them less promising material for spectrophotometry. We also used cell suspensions of *Dictyostelium discoideum*.

Light-minus-dark difference spectra were measured with a single-beam spectrophotometer on line with a PDP8/I computer. The absorption spectrum of the sample was measured (20 s per scan) just before and 30 s after a 30 s irradiation with actinic light obtained with a xenon lamp in conjunction with interference filters (12 nm half-bandwidth). The same timing sequence was used between irradiation and measurement in all experiments. The kinetics of decay of the light-induced absorbance changes after irradiation were measured with an Aminco-Chance dual-wavelength spectrophotometer.

The light-minus-dark difference spectrum (that is, the difference between the absorption spectrum measured after irradiation and that measured before irradiation) of a fresh

sample of *P. blakesleeanus* due to irradiation with 470 nm actinic light shows bleaching at 445 nm and an increase in absorbance at about 430 nm (Fig. 1a). If the sample was allowed to become anaerobic in the cuvette before irradiation, there was no bleaching at 445 nm but the absorbance increase at 430 nm remained (Fig. 1b). Both these absorbance changes decayed in darkness with a half-time of about 70 s.

Blue light at about 470 nm contains the most effective wavelengths for eliciting these absorbance changes. These wavelengths are also the most effective for the physiological blue light responses of *P. blakesleeanus*². Dense mycelial mats, however, are not suitable for measurement of an action spectrum of the photosensitive absorbance change; the response is not sufficiently stable for repetitive excitation of a given sample with different wavelengths and the preparation techniques are not sufficiently reproducible to permit fresh samples for each actinic wavelength.

Berns and Vaughn⁹ reported different light-induced absorbance changes with mycelial mats of *P. blakesleeanus*: irradiation with 345 nm actinic light caused an absorbance change between 460 and 345 nm (which could have been due to bleaching at 345 nm or to an increase in absorbance at 460 nm) of about 0.04 which persisted for several minutes. We tried to reproduce these measurements using a filter double-beam spectrophotometer comparable with that used by Berns and Vaughn, but found no light-induced absorbance differences between 460 and 345 nm greater than the noise level of the measurement (0.002).

Cell suspensions of *D. discoideum* were also examined for light-induced absorbance changes. The light-minus-dark difference spectrum of a fresh sample of cells taken near the end

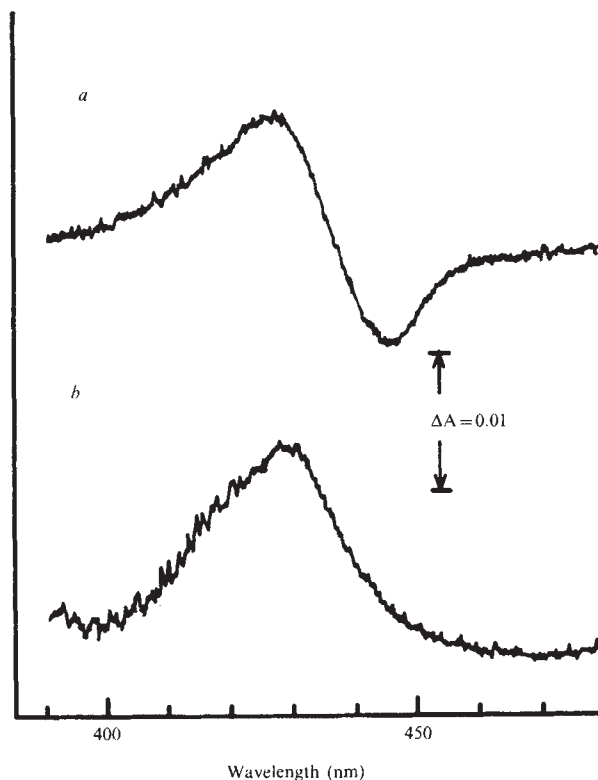


FIG. 1 Light-induced absorbance changes in *P. blakesleeanus*. A carotenoid deficient strain of *P. blakesleeanus* (C-2) was grown for 5 d in stationary culture after inoculation with heat-shocked spores of the medium described by Carlile¹³. The mat of mycelium was lifted from the culture flask and plugs were cut with a cork borer to fit the bottom of a cylindrical vertical cuvette. The sample was about 3 mm thick and represented an absorbance of about 3 at 425 nm. Actinic light was 3 mW cm⁻² at 470 nm. *a*, Light-minus-dark difference spectrum of a freshly prepared sample. *b*, Light-minus-dark difference spectrum of a sample permitted to go anaerobic in the cuvette before the measurements.

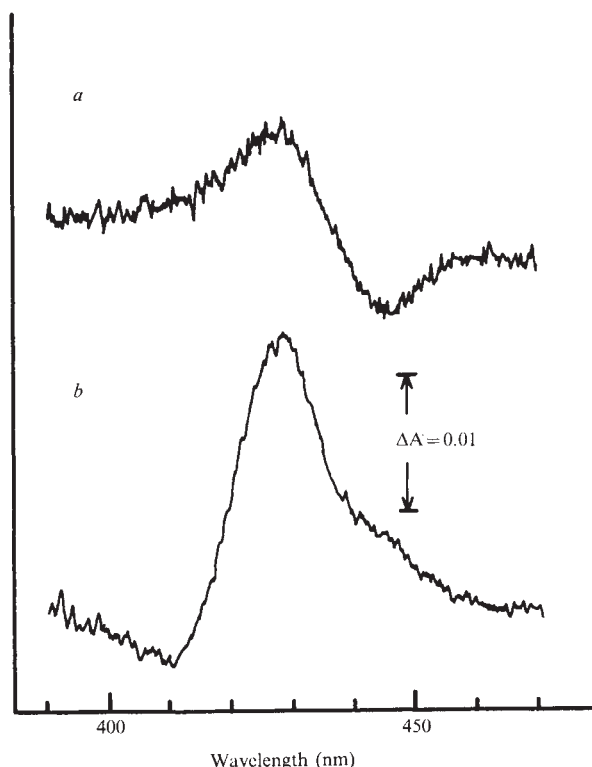


Fig. 2 Light-induced absorbance changes in *D. discoideum*. A strain A3 of *D. discoideum* able to grow in axenic culture was grown in liquid medium and collected as previously described¹⁰ while in late exponential growth phase. The sample consisted of a cell suspension (4×10^9 cells ml⁻¹) approximately 3 mm thick which represented an absorbance of about 3 at 425 nm. *a*, Light-minus-dark difference spectrum of a freshly prepared sample. *b*, Light-minus-dark difference spectrum of a sample permitted to age for several hours before the measurements.

of log phase growth (Fig. 2*a*) shows bleaching at 445 nm and an increase in absorbance at about 430 nm. If the cells aged for several hours before the experiment, there was no bleaching at 445 nm and the increase in absorbance at 430 nm was generally somewhat greater than without ageing (Fig. 2*b*). In the latter case, slight bleaching was also observed near 410 nm. The light-induced absorbance changes in cells of *D. discoideum* (Fig. 2) were essentially the same as those in the mycelium of *P. blakesleeanus* (Fig. 1).

Two different light-induced absorbance changes can be measured in cells of *D. discoideum*: one is a small rapidly decaying absorbance increase at 410 nm which can be induced by wavelengths as long as 600 nm¹⁰; the other is a larger, more slowly decaying absorbance increase at 430 nm (Fig. 2) which is induced only by wavelengths shorter than 520 nm. The small absorbance increase (about 0.001) at 410 nm, induced when cells of *D. discoideum* are irradiated with blue as well as yellow light, did not contribute to the difference spectra shown in Fig. 2. The small absorbance change decays in the dark with a half-time of 7 s and thus decayed to a negligible level during the 30 s between the irradiation and the start of the spectral scan. The light-induced absorbance changes shown in Fig. 2 for *D. discoideum* cells decay in the dark with a half-time of about 70 s, as did the similar changes in *P. blakesleeanus*, and thus persist long enough to be measured by the spectral scan after irradiation. The pigment which responds to the longer wavelengths of light has been extracted from *D. discoideum* cells and purified as a high molecular weight haem protein¹¹. This pigment appears to be the photoreceptor pigment for the photoactive response of *D. discoideum*. The pigment which responds only to the shorter wavelengths of light ($\lambda < 520$ nm) has been observed

both in cells of *D. discoideum* (Fig. 2) and in the mycelium of *P. blakesleeanus*. Its physiological function in *D. discoideum* is unknown (there may be photoresponses controlled by the blue-photoreceptor pigment which we are not aware of in *D. discoideum*) but it is a promising candidate for the blue-photoreceptor pigment which is manifest in so many different organisms.

An approximate action spectrum for the light-induced absorbance change in aged cells of *D. discoideum* (Fig. 2*b*) was determined by measuring the absorbance change induced by equal intensities (3 mW cm⁻²) of monochromatic actinic light. A plot of the magnitude of the light-induced absorbance change as a function of the wavelength of actinic light (Fig. 3) shows that the action is maximal at about 465 nm and absent beyond 520 nm. This action spectrum for the light-induced absorbance increase at 428 nm is consistent with those for the various physiological responses which are attributed to the blue-photoreceptor pigment.

If cells of *D. discoideum* are broken gently in a Dounce homogenizer, the photoresponsive pigment sediments with the mitochondrial fraction collected by differential centrifugation between 6,000 and 10,000*g*. If the cells are broken more thoroughly by passage through a Ribi press, the photoresponsive pigment is found in the soluble supernatant from a 1 h centrifugation at 144,000*g*. The absorption spectrum of the soluble extract after cell breakage in the Ribi press (Fig. 4, curve D) shows primarily reduced cytochrome *c*. The spectrum measured after irradiation (curve L) shows small light-induced changes which are seen best at higher sensitivity in the L-D difference spectrum. The light-induced absorbance changes in the soluble extract include an absorbance increase in the 560 nm region and a larger, sharper increase at 425 nm, both indicative of a photoreduction of a *b*-type cytochrome.

The photoactive pigment system apparently consists of a photoreceptor pigment, possibly a flavin, which absorbs maximally at about 465 nm, and a photoresponsive pigment, a *b*-type cytochrome, which increases in absorbance maximally at 428 nm *in vivo* and at 425 nm *in vitro*. In the fresh tissue the photoreceptor pigment may also induce bleaching at 445 nm which could be due to the oxidation of an *a*-type cytochrome. The proposed flavin-mediated photoreduction of a *b*-type cytochrome in soluble extracts suggests a cyto-

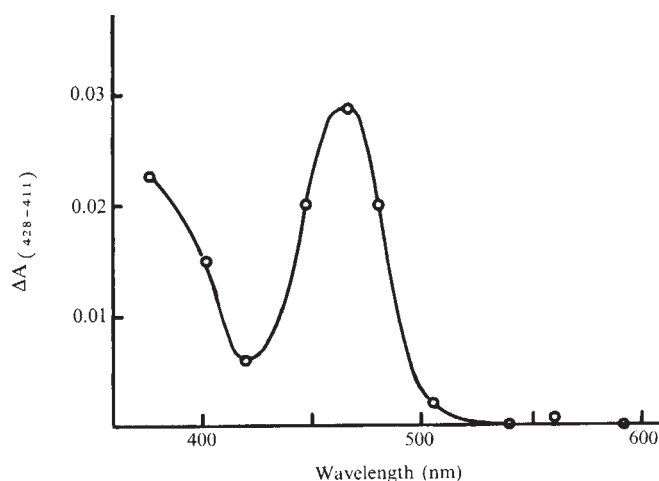


Fig. 3 Action spectrum for the light-induced absorbance change in *D. discoideum*. Aged samples were prepared as described in Fig. 2. The cells were irradiated with equal energies (3 mW cm⁻²) of monochromatic actinic light and the light-minus-dark difference spectra were recorded for the different wavelengths of actinic light. A fresh sample of cells was used for each actinic wavelength since the response tends to decay with repeated excitation. The magnitude of the light-induced absorbance change measured between the peak at 428 nm and the minimum at 411 nm is plotted as a function of the wavelength of actinic light.

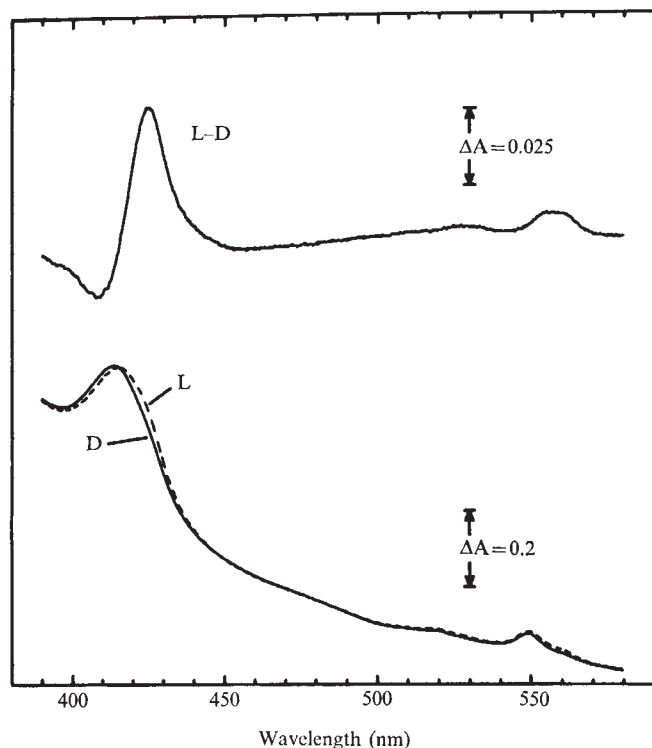


FIG. 4 Light-induced absorbance change in a cell-free extract of *D. discoideum*. Cells were grown as described in Fig. 2 to maximum stationary phase of growth at 1.2 to 1.6×10^7 cells per ml, washed, and concentrated to 10^8 cells per ml by centrifugation. The cells in this suspension were broken in a Ribi press at $12,000$ pounds inch $^{-2}$ and the subcellular particles eliminated by centrifugations at $1,000g$ for 5 min and $12,000g$ for 30 min. The soluble supernatant was then collected from a centrifugation at $144,000g$ for 60 min. Spectra of this soluble supernatant were measured at 22°C in darkness (D) and immediately after exposure to 3 mW cm^{-2} actinic light at 470 nm (L). The light-minus-dark difference spectrum (L-D) is also presented at an eight-fold increase in sensitivity (plotted directly by the computer).

chrome b_2 -like pigment with the flavin and haem prosthetic groups on the same protein molecule¹². These speculations remain to be confirmed but the experimental system is available.

We have not obtained a cell-free system from *P. blakesleeana* which shows the photoinducible absorbance changes but these experiments are continuing. We believe, however, that a common blue-photoreceptor pigment is present in widely diverse types of cells and that cells of *D. discoideum* may be a fortuitous source of the pigment.

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Actin in the alga, *Chara corallina*

ACTIN^{*} and myosin from muscle combine to form filaments with a distinctive arrowhead appearance¹. Filaments from several lower organisms²⁻⁵ and from cells other than muscle in higher animals⁶⁻⁹ also react with muscle myosin or its subfragments to produce arrowhead filaments. This ability to bind muscle myosin has become accepted as a test for the identification of the filaments as actin. Its validity is supported by the chemical similarities between those actins which have been further characterised^{5,6,8-13}, and by the need for the myosin binding sites to be quite specifically positioned and oriented for the arrowhead effect to arise¹⁴. A filament reacting with heavy meromyosin subfragment one (S₁) has now been detected in cells of the green alga, *Chara corallina*.

Internodal cells about 70 mm in length were centrifuged in a bench centrifuge at $600g$ for 5 min. Such treatment

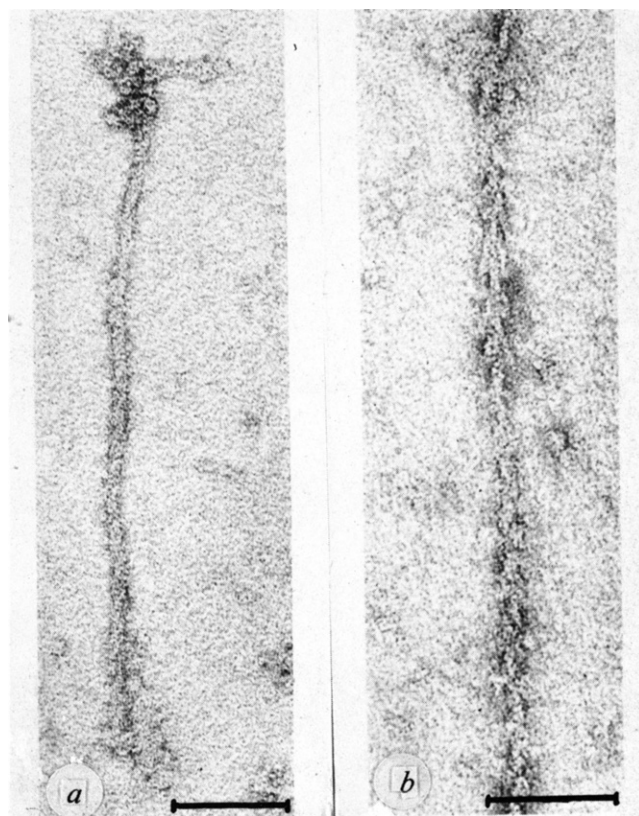


FIG. 1 a, A simple filament without S₁ treatment or, as in this case, after treatment with S₁ and ATP. b, Filament after treatment with S₁. In places an arrowhead effect is seen; elsewhere the surface of the filament is irregular. Bar = 100 nm .

displaces the flowing endoplasm to the centrifugal end of the cell, which can then be cut off and discarded. The remainder of the cell (that part containing stationary cortical cytoplasm only) was squeezed to expel its contents. The contents of 12 such centrifuged cells were pooled and spun at 200g for 2 min. The pellet was resuspended in 50 μ l of 150 mM KCl, 10 mM trisodium EDTA, buffered at pH 7.0 with 10 mM morpholinopropane sulphonic acid. After 10 min at room temperature, the preparation was again spun at 200g for 2 min and the pellet discarded. In some experiments, an S_i preparation from rabbit skeletal muscle was added either at the resuspension stage or to the final supernatant. The mixture was then kept at room temperature for between 10 and 60 min. All preparations were negatively stained with 1% uranyl acetate on carbon-coated specimen grids.

In the absence of the S_i preparation, the supernatant contained simple filaments of the type shown in Fig. 1a. After S_i treatment, filaments were found which in places had the distinctive arrowhead appearance of the actomyosin complex (Fig. 1b). If 1 min before transfer to the grid, neutralised ATP was added to a final concentration of 1 mM, the arrowhead effect disappeared (Fig. 1a). No arrowhead filaments could be found when the S_i preparation was examined alone.

Compared with the results obtained in parallel experiments with rabbit actin, the algal filaments showed, in so far as it could be judged, less regular arrowheads. Lengths of filament without arrowheads showed an irregular, roughened surface (Fig. 1b), again quite distinct from their appearance without S_i or after ATP treatment. Moreover, whereas stored S_i retained its ability to decorate rabbit actin, fresh S_i was needed to give convincing decoration of the algal filaments. These two observations may be a reflection of differences in S_i -binding ability between the algal and rabbit filaments, although further studies will be needed to establish this.

For the reasons summarised above, the formation of such arrowhead complexes is a preliminary, but probably reliable indicator of the presence of actin. The present experiment does not settle its position or function in these cells. In this connection however, microfilament bundles are known in closely related algae¹⁵⁻¹⁷, as well as in the cells of higher plants¹⁸. Unpublished light and electron microscope observations of cells of *C. corallina* have shown that the microfilament bundles are retained with the cortical cytoplasm on centrifugation. These microfilament bundles are believed to be involved in the mechanism of cytoplasmic streaming, and could well be the location of actin within these cells.

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Photoeffects in a flavin-containing lipid bilayer membrane and implications for algal phototaxis

FLAVINS have been implicated as receptor pigments in various plant photoresponses, such as phototropism¹, phototaxis² and chloroplast movement³. It has not yet been possible to prove conclusively the flavin photoreceptor hypothesis in any of these cases, nor has a mechanism been demonstrated by which a flavin could convert a light stimulus into a physiological signal. For the light-induced motor response involved in phototaxis of *Euglena*, we have suggested an electrical type of stimulus transduction by a flavin receptor pigment embedded in a lipid matrix⁴.

We report here light-induced electrical effects in an artificial bilayer membrane of the Mueller-Rudin type⁵ that contains an amphipathic riboflavin derivative. Riboflavin tetrapalmitate was prepared according to the method of Yagi⁶. It was incorporated into an oxidised cholesterol bilayer⁷ by adding it to the membrane-forming solution at a ratio, in the bulk phase, of 1 flavin molecule to 10 cholesterol molecules. The solutions of the aqueous phases (phosphate and citrate buffers or KCl, all 0.1 M) were made up from triply distilled water and ACS grade chemicals. Illumination was with white light from a 500 W tungsten projector lamp and was passed through glass heat filters, at a maximum intensity of 4 kW

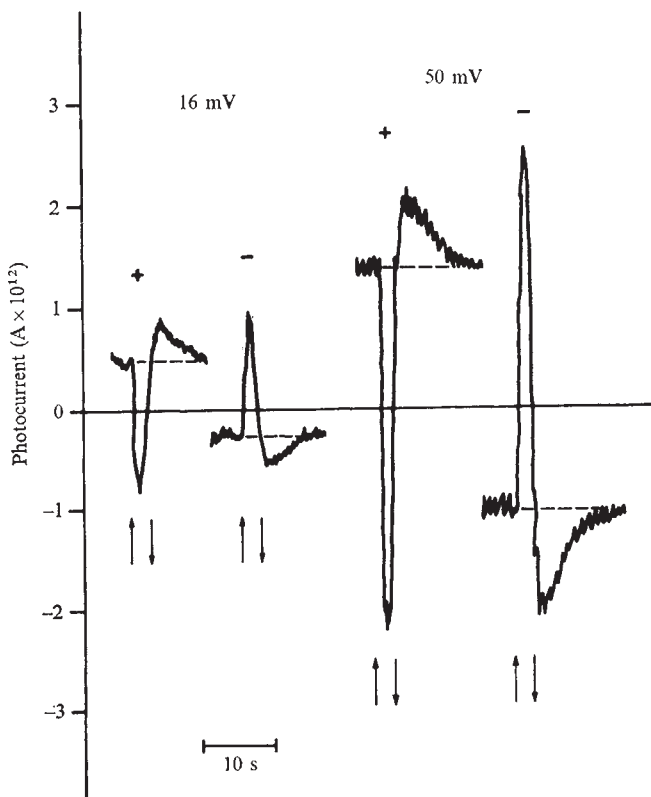


Fig. 1 Photocurrent observed as a function of applied potential on illumination of a bilayer lipid membrane containing riboflavin tetrapalmitate with white light (intensity 4 kW m⁻²).

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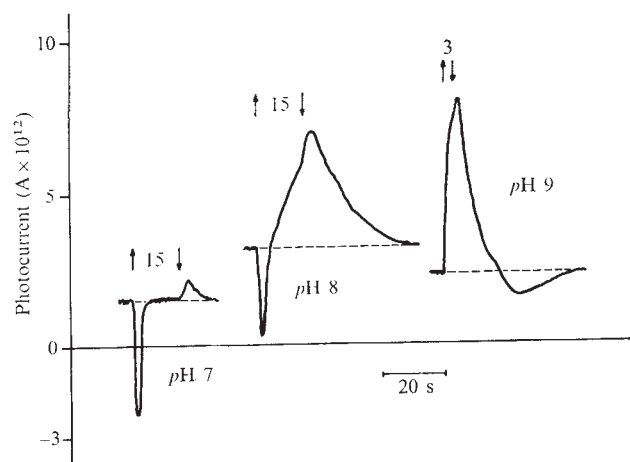


FIG. 2 Photoeffects in the flavin-bilayer membrane as a function of the pH of the aqueous phase.

m^{-2} ($4 \times 10^6 \text{ erg cm}^{-2} \text{ s}$). The photocurrent was recorded with a Honeywell Elektronik 16 strip chart recorder connected to a Keithley 610C electrometer.

Incorporation of the riboflavin tetrapalmitate did not affect the membrane resistance at neutral and slightly acidic pH, and decreased it by a factor of two to three at basic pH values. When normalised to the same peak height, photoresponses observed under the same conditions were very similar, though the magnitude of individual responses fluctuated greatly. We have not yet been able to pinpoint the reason for these variations.

As in all other systems studied so far, no photoeffects occur under completely symmetrical conditions. When an external potential was applied, at pH 6 and 7 we observed the effects shown in Fig. 1. The photoresponse consisted of a transient current decrease on illumination, followed by a return to the baseline and, when the light was turned off, a transient current pulse in the positive direction. This cannot be explained simply as an effect on membrane resistance, since the 'on'-current crosses the zero line and inverts direction transiently. The effect was the same in buffered and in unbuffered solution. From 16 to 70 mV, the peak heights of the response increased linearly with the applied potential.

As Fig. 2 shows, the nature of the photoeffect changed with increasing pH. At pH 8, the transient pulses when the light was turned on and off were still visible, but superimposed on this effect was an increase of the current lasting as long as the illumination, and slowly decreasing to the dark value after the 'off'-pulse had decayed. At pH 9 or higher, the negative 'on'-pulse was no longer discernible. In its place we observed a very rapid increase of current. The 'off'-pulse was still visible and followed by a transient slow decrease of the current below the dark value. At this pH, illumination for more than 5 s irreversibly decreased the membrane resistance.

Because of the above observation, and since the addition of flavins at pH values above 7 increases the current across the membrane even before illumination, we consider it

possible that the photoeffects appearing at basic pH values were, at least partly, due to effects on the membrane resistance. We have not yet attempted to substantiate this assumption.

We offer the following preliminary hypothesis to account for the characteristics of the photoeffects at neutral and slightly acidic pH. Illumination polarises the flavin moiety of the chromophore, perhaps through an electron acceptor/donor reaction of an electronically excited state of the flavin with another molecule in the lipid or aqueous phase. The polarised chromophore now interacts electrostatically with water dipoles at the membrane surface that had been pre-oriented by the applied potential. This reorientation of the dipoles corresponds to a transient transport of charges against the applied potential (Fig. 3).

This model accounts for the following observations. (1) The current pulses were transient, and a pulse in the direction of the applied potential appeared when the illumination was removed, that is when the reorienting force was released. (2) No photoeffect was observed without an externally applied potential, and reversal of the polarity of the applied potential changed only the sign of the photoresponse. (3) Increased applied potential increased the magnitude of the photoresponse, since more complete preorientation of the dipoles increased the number of charges which were transported on reorientation. At 70 mV, which was the largest potential we used because of membrane instability at higher

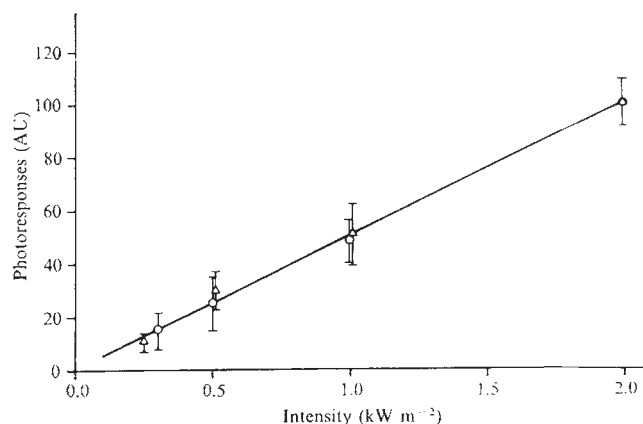


FIG. 4 Light intensity dependence of the flavin-bilayer membrane photoeffects at pH 7 (Δ) and the direct photophobic response of *Euglena* ($\circ$). The magnitude of both responses was arbitrarily set equal to 100 at the maximum intensity of 2 kW m^{-2} . AU, arbitrary units.

potentials, we had apparently not yet approached saturation effects caused by complete preorientation.

Calculations indeed predict that the triplet excited state of riboflavin tetrabutyrate should exhibit a dipole moment which is larger than that of the ground state by a factor of 1.47 (ref. 8).

Our model is similar to that proposed by Ullrich and Kuhn for cyanine dyes adsorbed to one side of a bilayer membrane⁹. It is probably incomplete in that it cannot account for the fact that the 'off'-pulse never reaches the expected same magnitude as the 'on'-pulse. One must assume that an irreversible reaction, probably a photodecomposition of the flavin, occurs in parallel with the dipole orientation process. In agreement with this reasoning, we have observed that under all experimental conditions the photoeffects become smaller when illumination is repeated.

The interpretation of our findings with respect to the mechanism of stimulus transduction in *Euglena* must be speculative, since we have as yet no *in vivo* electrophysiological data. We have suggested previously that in *Euglena*, a physiological signal is generated by means of photoconduction

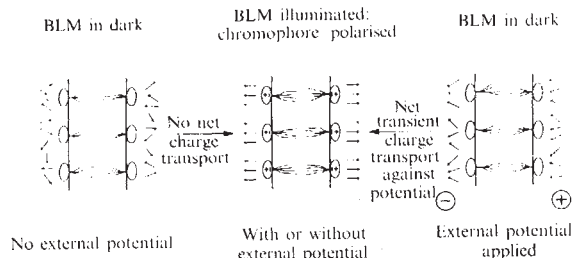


FIG. 3 Model proposed for the generation of the transient photocurrents at pH 7. BLM, bilayer membrane.

across a poised oxidation/reduction system. We observe in our bilayer membrane that photoeffects occur with an unbuffered ascorbate gradient across the membrane, but these effects disappear upon buffering. Apparently, the effects are in this case dependent upon a pH gradient rather than a redox gradient. In this context one might note that the acidities of the excited states of flavin have been calculated to considerably exceed that of the ground state¹⁰. Even though this prediction has not yet been tested experimentally, light-induced release of protons might thus provide an alternative mechanism for generation of the photocurrents observed by us.

The light intensity dependence of the bilayer membrane photoresponse at pH 7, and that of the direct photophobic response which *Euglena* exhibits when illuminated¹¹ are identical up to an intensity of 2 kW m⁻² (Fig. 4). This agreement may be fortuitous. At 4 kW m⁻² (a light intensity that we have not reached in measurements of *in vivo* responses of *Euglena*), the magnitude of the bilayer membrane photoeffect falls below an extension of the straight line in Fig. 4.

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Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid

L-GLUTAMIC acid is probably an excitatory transmitter of major significance in the mammalian central nervous system¹. The L-glutamic acid molecule is relatively flexible, and in an attempt to gain some insight into its possible shape(s) during activation of receptors associated with excitation of central neurones, a study was made of four conformationally restricted analogues: ($\pm$)-*cis*-1-aminocyclohexane-1,3-dicarboxylic acid ('cyclo-glutamic' acid), ibotenic acid, kainic acid and its dihydro derivative.

Ibotenic acid, an isoxazole isolated from the mushroom *Amanita muscaria*², and kainic acid, a pyrrolidine derivative isolated from the seaweed *Digenea simplex*^{3,4}, have previously been shown to excite central neurones^{5,6}. 'Cyclo-glutamic' acid is an inhibitor of glutamine synthetase⁷. In each of these cyclic compounds the ring restricts rotation about bonds equivalent to the C α -C β and/or C β -C γ bonds in L-glutamic acid.

The experiments were carried out on spinal interneurons and Renshaw cells of eight cats anaesthetised with pentobarbitone sodium (35 mg kg⁻¹ given intraperitoneally), administering the amino acids electrophoretically from seven barrel micropipettes⁸. Kainic acid, DL-homocysteic acid and L-glutamic acid were purchased from Calbiochem (Los

Angeles). Dihydrokainic acid was prepared by catalytic hydrogenation of kainic acid³ and N-methyl-D-aspartic acid was prepared by methylation of D-aspartic acid⁹. The micropipettes contained the following solutions: 'cyclo-glutamic' acid (1 M, adjusted to pH 7.3 with NaOH), dihydrokainic acid (0.2 M, pH 7), L-glutamic acid (0.5 M, pH 8), DL-

TABLE 1 Approximate potency ranges of excitant amino acids relative to L-glutamic acid, based on the ejecting currents required to achieve equal increases in firing frequency of spinal neurones

Amino acid	Potency range relative to that of L-glutamic acid
Kainic acid	8-80(12)*
N-Methyl-D-aspartic acid	7-18(3)
DL-Homocysteic acid	3-6(6)
Ibotenic acid	2-7(6)
L-Glutamic acid	1(25)
'Cyclo-glutamic' acid	0.7-0.8(3)
Dihydrokainic acid	0.06-0.6(5)

* Number of neurones tested.

homocysteic acid (0.2 M, pH 7.5), ibotenic acid (0.2 M, pH 7), kainic acid (0.1 M, pH 8) and N-methyl-D-aspartic acid (0.1 M, pH 8).

The approximate relative potencies (Table 1) of the amino acids as excitants of neuronal firing were assessed on the basis of results obtained with 17 interneurons and ten Renshaw cells. Kainic acid was more potent than N-methyl-D-aspartic acid, previously the most potent amino acid excitant reported¹⁰. On the other hand, dihydrokainic acid was the weakest excitant of the series. Figure 1 illustrates the effect of kainic acid on the firing rate of an interneurone in comparison with that of L-glutamic, DL-homocysteic and N-methyl-D-aspartic acids. The actions of kainic and N-methyl-D-aspartic acids were relatively slow in both onset and offset, compared to that of L-glutamic acid; this was also apparent with 'cyclo-glutamic', dihydrokainic and ibotenic acids.

An analysis of molecular models indicates that there is a unique juxtaposition of the equivalent ionisable groups in 'cyclo-glutamic', L-glutamic, ibotenic and kainic acids in the

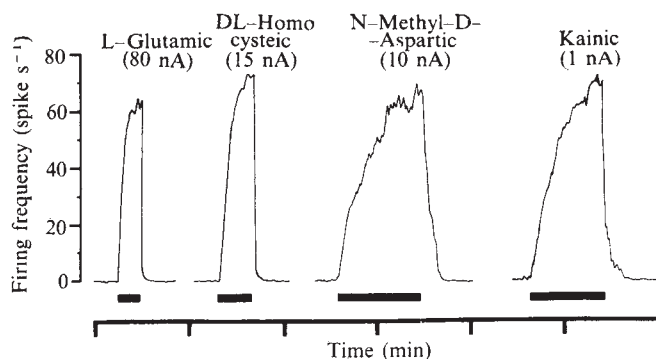


FIG. 1 Comparisons of the excitatory action of L-glutamic acid, DL-homocysteic, N-methyl-D-aspartic and kainic acids on a spinal interneurone. The black horizontal bars indicate times of electrophoretic ejection using the currents indicated (nA).

conformations illustrated in Fig. 2. It therefore seems possible that these amino acids interact with the same receptors. L-Glutamic acid is shown in a partially extended conformation with the C α -C β substituents eclipsed and the C β -C γ substituents staggered; this and the conformations shown for ibotenic and kainic acid would be expected to be relatively stable forms. The boat form of 'cyclo-glutamic' acid would be, however, less stable than a chair form. The

double bond in kainic acid must play a key role as saturation of this bond yields dihydrokainic acid which is much less potent than the parent compound as an excitant. This double bond, the conformation of which is arbitrarily illustrated in Fig. 2, might bind to a specific site on the receptor or might influence the preferred conformation and/or electronic properties of the neighbouring carboxyl group.

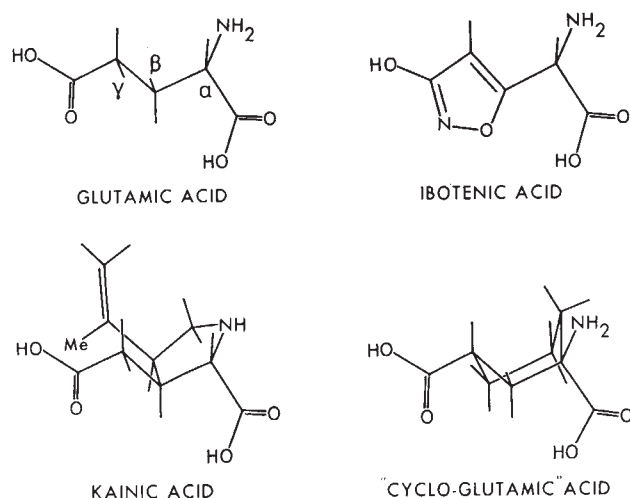


FIG. 2 Conformations of glutamic, ibotenic, kainic and 'cyclo-glutamic' acids in which the ionisable groups occupy the same absolute positions.

The ionisable groups of L-aspartic acid, also probably an important excitatory transmitter in the spinal cord¹, and of N-methyl-D-aspartic acid cannot match those illustrated in Fig. 2 for L-glutamic acid and its analogues. It is thus proposed that excitation by L-glutamic acid and L-aspartic acids results from preferential interaction by these two amino acids with different receptors. L-Glutamic acid might interact with 'glutamic acid preferring' receptors in partially extended conformations (approximating that shown in Fig. 2), and perhaps also with 'aspartic acid preferring' receptors in partially folded conformations. On the other hand, L-aspartic acid may interact only poorly, or not at all, with 'glutamic acid preferring' receptors because the carbon chain of L-aspartic acid is one atom shorter than that of L-glutamic acid.

The proposal provides one explanation of the differences in the relative potencies of L-glutamic and L-aspartic acids as excitants of Renshaw cells and dorsal horn interneurons¹¹. These neurones may have different populations of receptors for excitant amino acids, as a result of innervation of the interneurons, but not the Renshaw cells, by primary afferent fibres likely to release L-glutamic acid as a transmitter¹.

Further studies with conformationally restricted analogues of L-glutamic acid, which may have different affinities for 'aspartic acid preferring' receptors compared to L-glutamic acid, may aid in assessing whether there is overlap between 'glutamic acid preferring' and 'aspartic acid preferring' receptors, though the discovery of a selective antagonist for either receptor would be of more value.

Ibotenic acid and 'cyclo-glutamic' acids were gifts from Dr C. H. Eugster (Zürich) and Dr A. Meister (New York) respectively.

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Atmospheric pressure during incubation and post-hatch behaviour in chicks

DOMESTIC chicks are widely used as convenient experimental animals, but their use has a major drawback; one batch is frequently quite unlike another. Chicks hatching one week may, for example, be behaviourally sluggish whereas chicks hatching another week may be hyperactive. Striking differences have been found in a wide variety of behavioural and biochemical measures¹⁻³.

Some batch variation may be attributable to genetic differences in the parents but marked variability is still obtained when eggs are taken from the same flock. The quality of the eggs may change as the hens grow older but this factor could hardly explain the great fluctuations in responsiveness from one week to the next. Differences might be due to variation in the time between laying and incubation of the eggs. Batch differences, however, persist when this time is kept constant and all the eggs are kept at the same temperature before incubation.

The weather is, however, one factor which does change markedly and uncontrollably from week to week. While temperature and relative humidity are unlikely to be responsible for inter-batch variation, since they are kept within narrow limits during incubation, atmospheric pressure is not usually controlled and might be an important variable. I have therefore examined the association between atmospheric pressure and a variety of measures obtained in a long experiment on imprinting in which, for many weeks domestic chicks were incubated, reared and tested under the same conditions. The results of the analysis suggest that the chick embryo is, indeed, sensitive to atmospheric pressure at a certain stage in its development.

Ten batches each consisting of 90 fertile eggs from a broiler strain (Ross Chunkies) were collected at intervals from Ross poultry Products at Fornham All Saints, Suffolk. The eggs were incubated in a Western Turkeyette incubator kept at 37.5–37.7° C. The temperature was controlled by a sealed mercury contact thermometer. The eggs were transferred 18–19 d after the onset of incubation to a still-air Curfew incubator where they hatched. Throughout incubation eggs were kept in the dark and were in contact with other eggs^{4,5}. The chicks also hatched in the dark and were kept in a dark incubator until the time of the experiment. The mean post-hatch ages at the time of the experiments and the numbers of chicks used in each experiment are shown in Table 1.

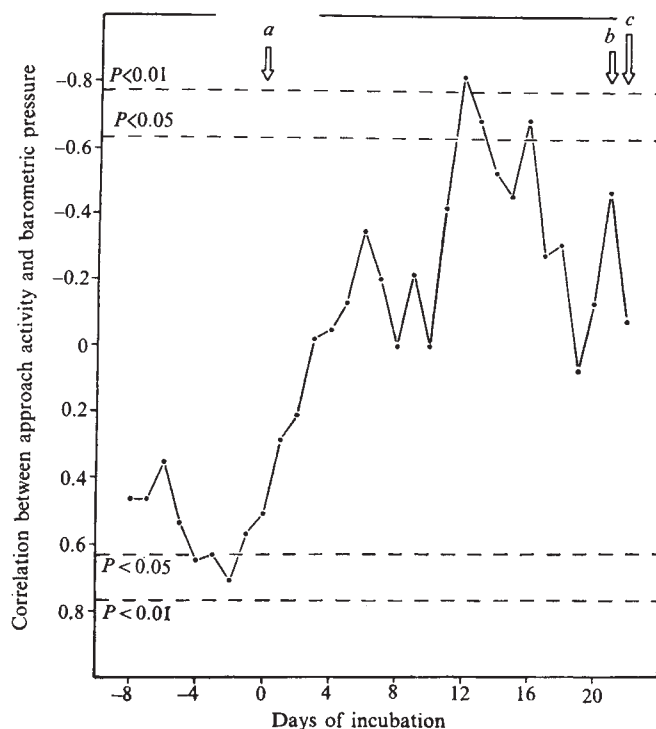


FIG. 1 The correlation between barometric pressure and the approach activity of day-old chicks plotted against the day of incubation from which pressure readings were taken. Spearman correlation coefficients are given. *a*, Onset of incubation; *b*, hatching; *c*, testing.

The chicks were placed in running wheels facing flashing, rotating lights⁶. Typically day-old chicks approach such a light after a short delay and do so with increasing vigour. They learn the characteristics of the light to which they are exposed and their social preferences are increasingly restricted to the familiar object (imprinting). In these experiments the chicks were exposed for a total of 72 min and later were given a choice between the familiar object and a novel one in apparatus which provided a quantitative measure of each bird's preference. The principal behavioural measures examined were: latency—the time taken from the beginning of the period of exposure to the moment when approach towards the flashing light began; approach activity—the counts of approach activity in the 20 min after each chick had first started to approach; and preference for familiar—the extent to which, in a choice test, the birds preferred the light

to which they had been previously exposed. Data for each batch are shown in Table 1. Noon barometric pressure readings were obtained from a meteorological station at Oakington, Cambs. which is 4.5 km from the laboratory and 13 m lower. During the period of the experiment the maximum recorded was 1,036 mbar and the minimum 984 mbar.

Barometric pressure was most strongly associated with approach activity. Figure 1 gives coefficients for correlations, between mean approach activity and barometric pressure, plotted against the day of incubation on which pressure was recorded. On the twelfth day of incubation, the correlation was negative and highly significant ($P < 0.01$); the lower the pressure, the more active were the birds when their approach scores were measured on the first day after hatching. The correlation was positive, though not as striking ($P < 0.05$), when pressure readings were taken from a few days before the onset of incubation. While this might be important, the pattern of weather at the time of the experiments was such that barometric pressure recorded 2 d before the onset of incubation was negatively correlated with pressure 12 d after the onset (Spearman $r_s = -0.697$, $P < 0.05$). Latency was not correlated with pressure at any time nor was preference for familiar. Another weather variable, relative humidity, which might conceivably be important, was not correlated at any time with approach activity. Hatchability, the mean of which was $84.52 \pm 1.90\%$, was not correlated with pressure. The median length of incubation was, however, positively correlated with pressure on the twelfth day of incubation ($r_s = 0.707$, $P < 0.05$); the higher the pressure, the longer the time taken from the onset of incubation to the peak period of the hatch. The length of incubation might have influenced activity since all the birds were tested at the same time in relation to the onset of incubation, and post-hatch age and approach activity are positively correlated ($r_s = 0.632$, $P < 0.05$). The activity of the chick embryos, however, could easily have affected their hatching behaviour. If the embryos were sluggish they would be expected to take longer over the highly energetic process of hatching. It seems likely that this was the case as the correlation between pressure and approach activity was stronger. But the matter can only be settled by direct experiment.

One qualification that must be made is that when a large number of correlation coefficients are calculated, a certain proportion of them would be expected to reach the arbitrary levels of statistical significance on a chance basis. Nevertheless it does seem probable that a causal link existed between atmospheric pressure in the second half of incubation and the chicks' subsequent activity. If so, what could the link have been? Possibly the slight effects of pressure on the concentrations of oxygen and carbon dioxide dissolved in the blood are

TABLE 1 Behavioural measures from ten different batches of domestic chicks.

Date of hatching (1973)	Number tested	Post-hatch age at experiment (h)	Latency (s)	Approach activity (Counts/20 min)	Preference for familiar (cm)
March 7	10	22.2 $\pm$ 0.3	174.8(76.9–817.7)	207.3 $\pm$ 54.8	22.9 $\pm$ 13.0
March 20	10	16.2 $\pm$ 0.5	79.1(20.7–140.2)	164.8 $\pm$ 39.2	7.1 $\pm$ 17.9
March 22	12	18.9 $\pm$ 0.2	203.7(60.2–346.9)	291.8 $\pm$ 66.2	13.6 $\pm$ 14.3
April 3	8	22.0 $\pm$ 0.4	110.1(76.1–389.0)	368.1 $\pm$ 76.9	6.8 $\pm$ 16.7
April 10	10	22.5 $\pm$ 0.2	192.9(116.5–354.7)	325.5 $\pm$ 73.1	18.5 $\pm$ 18.0
May 1	11	20.5 $\pm$ 0.5	176.1(30.6–268.6)	232.3 $\pm$ 37.5	9.5 $\pm$ 16.5
May 8	10	22.2 $\pm$ 0.2	337.4(218.0–683.9)	324.0 $\pm$ 69.8	16.8 $\pm$ 14.7
May 15	12	22.3 $\pm$ 0.2	185.6(100.0–416.1)	365.0 $\pm$ 55.0	2.8 $\pm$ 8.4
May 17	12	21.7 $\pm$ 0.2	219.0(162.9–398.8)	299.6 $\pm$ 60.2	–9.8 $\pm$ 13.0
May 22	11	20.2 $\pm$ 0.6	112.0(31.2–192.8)	216.9 $\pm$ 47.8	–2.2 $\pm$ 12.7

Means and standard errors are given except for latency for which medians and interquartile ranges are used. In measuring approach activity four counts were obtained for each revolution of the running wheel. The preference for the familiar was the maximum distance travelled from the midpoint between the familiar light and a novel one. Each chick was placed in a specially geared wheel in which the bird's movements carried it away from the object which it tried to approach. When a chick has been carried sufficiently close to the less preferred light, it usually turned around and attempted to approach that one⁷. A negative score means that a chick attempted to approach the novel stimulus.

critical at a particular stage in the development of the chicks' central nervous system. Hatchability is reduced when eggs are incubated at high altitudes and may be improved by increasing the partial pressure of oxygen⁷. The range of barometric pressure in the experiment reported here, however, corresponded to a difference in altitude of less than 450 m and, of course, pressure fluctuated. Furthermore no association was found between pressure and hatchability. The results certainly suggest that it would be worthwhile examining experimentally the effects of small transient changes in pressure on measures which are more sensitive than the percentage of eggs that hatch. Whatever the outcome of such analysis, the control of pressure may remove one of the irksome sources of differences between batches of chicks.

Behavioural and biochemical details of the experiment reported here will be published elsewhere. I thank Miss J. B. Jaecel for assistance and Drs. G. Horn, and R. Oppenheim, and Miss M. A. Vince for their comments on this manuscript. The work was supported by a grant from the Science Research Council.

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Nickel accumulation by *Hybanthus floribundus*

ACCUMULATOR plants are known for many naturally-occurring chemical elements¹. The term 'accumulator plant' is used here to describe plants which have dry-weight elemental concentrations greater than either the associated substrate or 'normal' plants. Much work has centred on copper², zinc³ and selenium⁴ accumulators but little attempt has been made to explain the rationale of elemental accumulation. It is usually considered a 'tolerance' mechanism⁵, and only recently has the functional or essential role of these elements been suggested⁶.

Hybanthus floribundus is an Australian shrub species which accumulates nickel and cobalt with modal levels of 5,000 p.p.m. and 100 p.p.m. respectively, in leaf tissue⁷. Two zinc accumulating *Hybanthus* varieties have also been discovered in the northern savanna zone of Australia.

H. floribundus embraces several sub-species or ecotypes, (to be fully described elsewhere), and has an insular or disjunctive distribution across the southern part of Australia. The three nickel accumulating ecotypes, however, are re-

stricted to a broad belt in Western Australia, which extends from Ravensthorpe near the southern coast, to Agnew, 450 miles to the north. One of these varieties (*H. floribundus* ssp. *curvifolius*) is the subject of detailed distribution and biogeochemical studies in the central part of the belt⁸. The habitat of this shrub is characterised by nickeliferous soils which are defined here by nickel levels in excess of 400 p.p.m. (water-soluble nickel varies around 10 p.p.m.) These soils are often lateritised and result in low calcium levels (less than 4,000 p.p.m.) and a pH range from 5.0 to 6.9. The associated plant species are xeromorphic *Acacia* and *Eucalyptus* trees, and *Eremophila* shrubs. The *Hybanthus* shrubs are conspicuous by their apparent lack of xeromorphic adaptation to a climate which becomes increasingly arid towards the continental interior. This feature has been noted by others⁸.

TABLE 1 Nickel concentrations in soil and various parts of *H. floribundus*

Entire plant	Nickel (p.p.m.)	
	Dry weight	Ash weight
Whole leaf	6,542	130,000
Photosynthetic stem	5,490	132,000
Lower woody stem	3,806	125,000
Peeled root	415	12,800
Root bark	221	4,200
Soil	770	780
Aerial organs		
Outer leaf tip	4,869	98,790
Petiole half of leaf	4,023	82,540
Photosynthetic stem	1,179	31,300
Hard inner wood	502	55,900
Soft outer wood	1,269	34,580
Trunk bark	1,653	26,690
Soil	620	630

Nickel concentrations in the various 'insular' populations of *H. floribundus* were generally observed to decrease southwards (from 8,000 to 1,000 p.p.m.) as the annual rainfall increased from 7 inches to more than 30 inches. These 'ecotypes' can be recognised sometimes on morphology alone, and always by the use of organic (amino acids) and inorganic chemotaxonomy. The principal discriminators are N-acetyl ornithine, nickel and cobalt⁹. A study of intraplant distributions for nickel and other significant elements, shows that nickel concentrations increase from the roots through the rootstock, into the stems and reach maxima towards the leaf tips (Table 1). High nickel concentrations are also seen in seed capsules (1,500 p.p.m.), viable seeds (2,000 p.p.m.) and flowers. The mean concentration of nickel and cobalt in flowers (47 samples) is 5,003 p.p.m. and 8.7 p.p.m. respectively, whereas corresponding leaf concentrations are 3,665 p.p.m. and 24.8 p.p.m. It is evident that nickel is not excluded from reproductive tissue.

The maximum nickel concentration recorded to date is 1.6% (26% nickel in ash) in mature leaf tissue and this is in agreement with Cole⁸. Other elemental concentrations in leaf tissue, given as the mean of 217 samples in p.p.m. dry weight are: chromium 3, copper 5, cobalt 20, zinc 30, manganese 100, iron 370, sodium 1,100, magnesium 3,320, calcium 3,920 and potassium 8,000. Conventional atomic absorption was used to monitor the various elements. At these concentrations it was possible to measure nickel accurately in leaf segments weighing as little as 1 mg; mature leaves typically weighed 7 mg.

The Pearson product moment correlation coefficient (r) indicated several significant interelement relationships (Table 2). From those which are significant, it is evident that calcium is the only element to correlate strongly with nickel. This highly significant inverse relationship is observed in

TABLE 2 Interelement correlations (r) in *H. floribundus*

	Leaf Tissue (74 samples)							
	Mg	Ca	Fe	Mn	Zn	Cr	Co	Cu
Ni	0.0163	-0.4504*	-0.0240	0.1815	0.2411	-0.1446	0.2579	-0.0043
Cu	-0.0618	-0.1903	0.3621*	0.1885	0.2187	-0.0077	-0.1192	
Co	0.2674	-0.0959	-0.0353	0.4085*	-0.1150	0.1102		
Cr	-0.0305	0.0486	0.5633*	0.2260	-0.0205			
Zn	0.0238	-0.4097*	0.0332	0.1744				
Mn	0.1528	0.1792	0.1007					
Fe	-0.0833	-0.2216						
Ca	-0.1732							
	Twig tissue (72 samples)							
	Mg	Ca	Fe	Mn	Zn	Cr	Co	Cu
Ni	0.1959	-0.6297*	-0.1025	0.1022	0.1241	-0.0915	0.0811	0.1037
Cu	-0.2055	-0.2116	0.0813	0.1911	0.2250	0.1306	-0.1889	
Co	0.2981	-0.1500	0.3807*	0.1041	-0.2140	0.3074*		
Cr	-0.1313	-0.0300	0.6699*	0.3859*	0.1345			
Zn	-0.0509	-0.0408	0.1580	0.2251				
Mn	-0.0293	0.1801	0.3407*					
Fe	0.0301	-0.0660						
Ca	-0.1130							

* Correlation significant, $P < 0.01$.

leaf, stem and wood tissue. Calcium was not measured in flowers but three significant correlations were observed, namely, nickel-manganese (negative), nickel-cobalt and cobalt-manganese. The effect of the inverse nickel-calcium relationship is to maintain the nickel plus calcium concentration at near constant levels in each of the three tissues examined. This suggests it is a substitution mechanism, and is supported by the recent findings of nickel in pectinates⁸.

More detailed studies, involving leaf tissue, indicate that nickel exists *in vivo* as a small (molecular weight less than 250) water-soluble cationic compound in agreement with a previous report¹. Histochemical staining (with dimethyl glyoxime) demonstrated a remarkable concentration of nickel in the epidermal layer which has been described elsewhere^{10,11}. The subcellular localisation of nickel was indicated to be at or near the epidermal cell walls, by means of an electron probe microanalyser¹². Leaching experiments were carried out in the glasshouse. These showed that simulated summer rain removed insignificant amounts (<0.5%) of nickel from aerial tissues, in spite of the epidermal concentration.

The accumulation of nickel in epidermal tissue of *H. floribundus* has been clearly demonstrated, but there is no evidence for a disposal mechanism. The leaves are perennial and nickel is not leached from them in significant amounts. This then raises the question, is nickel required by the plant? The essential role of nickel in *H. floribundus* was proposed⁷ on the basis of a statistical analysis of analytical data. Moore¹³ however commented that 'a physiological requirement for nickel' is difficult to explain. Cole⁸ listed the following alternatives to explain the causes of nickel accumulation by *H. floribundus*: (1) internal stress as the plant adjusts to more arid areas, (2) unrestricted transpiration and (3) a requirement for large quantities of nickel. A further suggestion¹³ is for localised selection pressure as the causal factor. There are, however, several features which can be merged to provide a hypothesis for nickel accumulation by *H. floribundus*. It is surely more than coincidence that the sole species in this arid region which does not display any apparent xeromorphic adaptation is also the only nickel accumulator. The regional correlation of increasing nickel concentrations with decreasing rainfall has been mentioned and I suggest that epidermal accumulation of nickel (where concentrations probably approach 5% or more, fresh weight) reduces cuticular transpiration and that in fact, nickel accumulation by *H. floribundus* is a xeromorphic adaptation. The origins of this idea are not new (see refs in ref. 14).

The need for nickel can account for the restriction of *H. floribundus* to nickeliferous soils in this arid environment. The present day disjunctive range of this species is the remnant of a former widespread distribution^{15,16} which retreated southwards with the onset of aridity. It is possible that the insular populations survived because they had evolved the ability to accumulate nickel, no doubt aided by polyploidy. Metal accumulation as a xeromorphic adaptation, may have application to accumulator plants in relatively arid regions elsewhere, and in particular to *Becium homblei*, the copper accumulator¹⁷ of south central Africa. Field experiments are now being set up in an attempt to establish the validity of this model. An initial approach will be to quantify the effect of nickel, cobalt and calcium on the drought resistance of *H. floribundus*.

I thank Dr R. R. Brooks and Professor P. J. Peterson for supervising early parts of this work, and also Drs G. A. Challis, P. F. Reay, A. Craig, W. D. Bennett and E. S. T. O'Driscoll for assistance.

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book reviews

Coral, the living rock

Biology and Geology of Coral Reefs. Edited by O. A. Jones and B. Endean. Vol. 1 (Geology 1): pp. xvi+410; vol. 2 (Biology 1): pp. xxi+480. (Academic: New York and London, November 1973 and January 1974.) Vol. 1: \$28; vol. 2: \$42.50; £20.40.

THE appearance of these two volumes, to be followed by a like number, indicates the present interest in coral reefs. The great controversies of the last century concerning their origin, unresolved by the outcome of expeditions to Funa-futi, were succeeded by a period of greater interest in living corals including taxonomy and the vexed significance of growth forms, work associated with the names of Mayor and Vaughan in the United States, of Stanley Gardiner in Britain and of Dutch workers in the then Netherlands Indies with the lone enquiring figure of Wood-Jones at Cocos-Keeling.

It was on the falling crest of this wave that in 1928 I took the Great Barrier Reef Expedition to Australia to study reefs as living systems but the only follow up came from the Tropical Biological Station established by the Japanese at Koror in the Palau Isles in 1935 and later destroyed in the war.

The present wave of interest was generated by mighty forces. In 1945 the United States Government decided to test atomic weapons at Bikini Atoll in the Marshall Isles. 'Operation Crossroads' in July 1946 was followed by other explosions until testing ceased in 1958. Accompanying operations involved major oceanographic surveys with wide ranging geological and geographical studies including the deep drillings on Eniwetok which, at long last, ended the controversy in Darwin's favour. Most detailed analyses of coral formations included taxonomy and ecology of corals.

But although attempts were made to estimate productivity, there was a singular lack of what may be described as intimate contact with living corals. This came with the work of T. F. Goreau in the Caribbean. Starting with calcification which he studied experimentally *in situ* below the surface, he proceeded with ecological analyses of coral formations and also revealed the unexpected richness of the Atlantic coral fauna. The greatest blow that coral reef studies have suffered was his premature death in 1970.

The editors of these volumes which summarise much of what has been achieved are connected with the Great Barrier Reef Committee at Brisbane which maintains an active centre for teaching and research on Heron Island in the Capricorn Group. When completed by the publication of two further volumes, the entire work will contain 46 contributions by 41 authors from 6 different countries. Unfortunately there is no indication of the contents of these future volumes so that one cannot judge how complete will be the final coverage.

There is little arrangement of chapters and no cross references between them; each must be read in isolation; however one knows the difficulties inherent in such composite works. The first, geological, volume which is essentially descriptive, contains an excellent account of Caribbean reefs by J. D. Milliman. His comparisons between these and Indo-Pacific reefs are illuminating and his description of sand cays as "slowly moving sand waves" is apt. There is no one so well qualified as Harry Ladd to write about Bikini and Eniwetok Atolls and everything affecting corals that occurred there. All will be grateful for this comprehensive statement.

The extensive recent work on reefs in the Indian Ocean, including Aldabra, which was covered by the symposium in 1971 is well summarised by David Stoddart. The reefs of French Polynesia (The Marquesas, Tuamotus, Society Islands, and the Austral Archipelago) with those of New Caledonia are described by J. P. Chevalier. F. W. Whitehouse deals with the reefs off New Guinea but strangely without illustrations: the one map referred to was certainly not in the copy I have received. Not surprisingly the volume ends with five chapters devoted to various aspects of the Great Barrier Reef, from the nature of its waters to geomorphology, structural and tectonic factors and sediments. There is a mass of useful information here—asking to be coordinated some day—although the maps illustrating distribution of sediments, some of them appearing isolated at the end of the book, are very difficult to understand; colour should have been used.

Much is made in the biological volume of microbial ecology and the pos-

sible—for it is as yet no more—significance of the microflora in coral nutrition. In a later chapter on marine antibiotics, interesting enough in itself, there seems to be no specific reference to scleractinian corals, only to gorgonids which are now, at any rate in the Caribbean, assuming unexpected significance as a source of prostoglandins.

Leonard Muscatine gives an expectedly judicial survey of the nutritional problem in hematophytic corals. But his extensive references to our work in 1928/29 is clear indication of how little, apart from studies on the zooxanthellae, to which he has contributed so notably, has since been done. J. H. Connell's chapter on coral ecology is also to be commended. He stresses the significance of interactions between coral colonies, a further indication (so badly needed) that, despite the immobile masses of lime they form, corals are living animals.

Peter Glynn contributes a long descriptive account of the ecology of coral reefs in the Caribbean which can usefully be read with Milliman's chapter in the other volume. G. J. Bakus provides what I take to be the fullest review to date of knowledge about tropical holothurians, so numerous in species and so ubiquitous on sandy areas of reefs. The last two chapters deal with that most destructive echinoderm, *Acanthaster planci*, adding to the rich literature on this now notorious, but biologically so interesting, starfish.

These volumes contain a wide range of information, the chapters inevitably varying widely in degree of relevance and mode of approach. They will be of real value to all engaged in aspects of coral reef research although they are hardly likely to draw anyone to the subject. They lack the breath of recent research which permeates the pages of the just completed "Coral Reef Project. Papers in Honor of Dr Thomas F. Goreau 1924-1970" (*Bull. mar. Sci.*, Miami, vol. 23) which should be seen by all who are interested in the volumes under review. The two further volumes will soon, one hopes, be available. The editors somewhat strangely dedicate the work in part to themselves but, more significantly, add the name of H. C. Richards, at one time Professor of Geology at Brisbane, to whom I gladly offer a personal tribute.



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Studies on Excitation and Inhibition in the Retina

EDITED BY F. RATLIFF

April 1974: 688 pages: £8.50

For nearly half a century, Nobel Laureate H. Keffer Hartline has conducted research on vision and the retina. His studies have elucidated numerous fundamental principles of retinal physiology which, over the years, have provided the foundations for many advances in the neurophysiology of vision. This collection of papers from Professor Hartline's laboratory, together with the specially written introductory sections, provide for the first time a full account of the development of this subject, and show how Hartline's particular mathematical-experimental approach is being adopted widely in the analysis of complex neural networks.

Plant Viruses

K. M. SMITH F.R.S.

Fifth edition, March 1974: 224 pages: £3.30 Science Paperback £1.30

This new edition has been expanded to cover the present knowledge on the whole field of plant viruses. So far as it is possible in a book of this size, all the latest developments in the subject are included, such as the assembly of some virus particles, the discovery of the 'viroids', the latest information on the transmission of viruses by fungi and the most recent work in the way that viruses affect the fungi and also the algae.

Telecommunications

J. BROWN

Second edition, 1974: 400 pages £5.50 Science Paperback £3.00

A Review of the Principles of Electrical and Electronic Engineering

To be published in four volumes
EDITED BY L. SOLYMAR

Volume 1: Principles of Heavy Current Engineering

February 1974: 124 pages: £1.65.

Volume 2: From Circuits to Computers

February 1974: 192 pages: £2.50

Further details on these titles and a list of stockists available from the publishers on request.

Colonial animals

Animal Colonies: Development and Function Through Time. Edited by Richard S. Boardman, Alan H. Cheetham, and William A. Oliver, jun. Pp. xii+603. (Dowden, Hutchinson and Ross: Stroudberg, Penn.; Wiley: Chichester, December 1973.) £17.50.

THIS book, the outcome of a symposium held in Washington in 1971, consists of a series of papers which attempt to analyse the basic nature of metazoan colonies and to provide new information on the development and function of key groups of colonial animals, with emphasis on those with good fossil records. The various papers illustrate two common themes as regards the definition of coloniality: members of the colony must be physically connected and they must have common ancestry through a sexual reproduction; social insects and certain 'colonial' forms are therefore excluded. The assumption of genetic constancy allows a direct evaluation of other factors controlling morphological and physiological differences within the colony, such as ontogeny, polymorphism and environment.

Attention is restricted to four groups of animals, the coelenterates, bryozoans, graptolites and sponges (whose interpretation as solitary or colonial animals remains controversial). The treatment of these groups, however, can hardly be described as balanced. Thus the bryozoans cover 42% of the papers and 55% of the pages whereas the unfortunate sponges get only 11% of the papers and 6% of the pages.

With regard to the individual contributions of authors, it is difficult to make generalisations, because they range from lengthy, comprehensive reviews to short morphological, physiological or ecological accounts of individual species or even, in a couple of cases, to one page abstracts. Most of the material can only be adequately evaluated by a specialist in the particular groups concerned, but several papers stand out as of more general interest. For instance, Coates and Oliver examine the kinds and levels of integration in fossil and living zoantharian corals and discuss the evolution of coloniality in relation to the formation of reefs. Boardman and Cheetham, in the longest paper in the volume, evaluate the pattern of increasing structural and functional integration of bryozoans through geological time, and infer the operation of mosaic evolution. Schopf propounds and confirms to his satisfaction a model relating the degree of polymorphism in bryozoans to stability of the environment. Urbanek discusses the organisation of graptolite colonies, which are interpreted in terms of physiological gradients and genetics and in

the broader context of evolution. Although the book as a whole has a palaeontological bias there is a full coverage of living organisms and some papers deal exclusively with these.

Illustrations are abundant, with 140 line drawings and more than 200 photographs, and the production is good. The book can be recommended to all professional palaeontologists and zoologists interested in colonial animals.

A. HALLAM

Iron to carry electrons

Iron-Sulfur Proteins. Vol. 1: Biological Properties; vol. 2: Molecular Properties. Edited by Walter Lovenberg. Vol. 1: pp. xiii+385; vol. 2: pp. xiii+343. (Molecular Biology: An International Series of Monographs and Textbooks.) (Academic: New York and London, November 1973 and January 1974.) Vol. 1: \$33; vol. 2: \$29.

THE excitements and developments during barely two decades of research on iron-sulphur proteins make compulsive reading for those in the field and possibly to those who are entering it—and perhaps also to students who want to find out how research has been pursued and what knowledge has been accumulated now (or rather, about two years ago). The name iron-sulphur proteins applies to iron proteins in which the iron is liganded to sulphur—it is non-haem iron. The iron-sulphur proteins seem to be ubiquitous from the "primitive", obligate, anaerobic bacteria to higher plants and animals. They mainly function as electron carriers at very low redox potentials (although this can be altered) in most of the principle reactions sustaining life such as photosynthesis, nitrogen fixation and electron transport in subcellular membrane systems.

Volume 1 contains an excellent chapter on nomenclature and history by H. Beinert which puts the field in perspective and shows how an apparently new field has been based on unrelated endeavours in three fields: photoreactions in chloroplasts, previously unaccounted for protein-bound iron in mammalian systems, and nitrogen fixation in bacteria. Now that non-destructive and specific methods of analysis such as ESR and ENDOR (with rapid freezing techniques), NMR, and Mössbauer spectroscopy are available for studying the iron-sulphur proteins (which are much more labile than, for example, cytochromes) their structures and roles are being reported frequently and the field is spreading rapidly into many aspects of biochemistry and molecular biology.

The other nine chapters of volume 1 deal with the properties and roles of

iron-sulphur proteins in diverse reactions of bacteria, nitrogen fixation (where model compounds with biological activity are being extensively pursued), photosynthesis, carbon fixation, mono-oxygenase reactions, hydrocarbon hydroxylations, steroid hydroxylation in adrenals, and flavoprotein dehydrogenases and hydroxylases.

The wealth of information now available on iron-sulphur proteins and their roles in the above types of reactions is very well presented by the authors and well edited by Lovenberg to minimise overlap. There is a comprehensive subject and author index.

For the second volume, suffice it to quote the editor's preface: it "provides an in-depth analysis of the chemical and physical properties of many of the iron-sulphur proteins. Particular emphasis is placed on the theory and use of physicochemical techniques in the study of metalloproteins".

This volume thus provides the important companion to the first where biological properties were discussed. The importance of the biological integrity of the protein when its physicochemical properties are being investigated is rightly stressed, as this point has often been neglected in the past when physical techniques have been applied to biological compounds. This is especially important with the generally quite labile active centre of iron-sulphur proteins.

There are fascinating sections on the evolution and genetics of iron-sulphur proteins, how to build up the X-ray structure of rubredoxin, the theory and practise of EPR and ENDOR, and many comprehensive tables of useful data.

It is such a pity that it inevitably takes so long for a book to reach the desks of researchers in such rapidly developing fields; but there is now a comprehensive summary by specific workers in the field up to 1972 (addenda have been added to a number of chapters in both volumes)—and one must be thankful for this! The cost of the two volumes is very high but I hope that this will not deter anyone from having the books constantly at hand whenever referring to iron-sulphur proteins.

D. O. HALL

The tektite mystery

Tektites. Edited by Virgil E. Barnes and Mildred A. Barnes. Pp. xv+445. (Benchmark papers in Geology.) (Dowden, Hutchinson and Ross: Stroudberg, Penn.; Wiley: Chichester, 1973.) £10.

As if problems recognised to be geological were not numerous enough, tektites seem to present an entirely separate mystery. Strewn fields of these strange objects are known at eight restricted

sites scattered over the globe. They mostly weigh tens of grams, and consist of highly refractory compounds, about 80% silica for example. The oldest are the bediasites at about 40 million years, and the australites youngest at under a million years. They bear little discoverable relation to their surroundings, yet they are found practically where they must have originally fallen from the sky.

Held together by surface tension just before they finally come through the high atmosphere, the tektites are there distorted into numerous aerodynamic forms: some australites starting quite spherical ablate to characteristic button-like shapes, which can and have been precisely reproduced in wind-tunnels. Remarkable indeed is the property that within every cubic centimetre of a tektite are embedded hundreds of micron-sized intrusions, while minute occluded gas bubbles are at pressures of order only a thousandth of an atmosphere.

This book consists of 45 individual papers, grouped by topics each with introductory editorial comments, and provides a volume of fascinating interest. It must cover everything known and conjectured about tektites, except perhaps a satisfactory answer to the great problem of their origin. Possible relevance to solving the re-entry problem (for spacecraft) intensified their study at that time, and for a decade a source from meteoritic lunar impacts was widely supported as the most likely answer. But the moon has since been more closely examined, with nothing much hopeful for tektites found thereon, and a terrestrial source now seems inescapable.

But they cannot result immediately from impact, and the only possibility would seem that they represent a far more remote end product of terrestrial meteoritic impacts, of sufficient violence to gasify huge amounts of surface rocks and project a vigorously expanding cloud from the resulting explosion. In cooling, the most refractory substances would condense out, and sprayed in all directions, their trajectories round the Earth would converge again to form a heated and melted accretion stream descending at the antipodal region. Fragmenting under terrestrial acceleration into solidifying droplets, these would continue to be bombarded for a time by further tiny solid particles converging to the stream.

All this may seem highly improbable, but it would explain why no tektites are found in the whole Soviet Union (one sixth the land surface) since this lies opposite much of the watery Pacific, whereas the known fields are all effectively antipodal to land masses. And if this (very briefly) is not how they were formed, one can only ask what other mechanism can be suggested?

R. A. LYTLETON

Stave off starvation

Nutritive Value of Triticale Protein (and the Proteins of Wheat and Rye). By Joseph H. Hulse and Evangeline M. Laing. Pp. 183. (International Development Research Center: Ottawa, 1974.) \$7.50.

THOSE of us who are concerned about the long-range prospects for world food supplies have for many years been grumbling that we depend for almost all our food on plant species selected by primitive man—or more probably woman. The first break with this unseemly dependence on the past comes, not from the domestication of a new wild species, but from the creation of a new species. This is triticale: a hybrid of wheat and rye (*Triticum x Secale*). Ostensibly, this is a book about it. Unfortunately, the authors have spread themselves over the whole subject of the properties and manner of use of wheat and rye, and the results of adding supplements such as amino acids, soya, fish and microbial products to them. Triticale gets rather lost; less than a third of the book deals specifically with it. Mainly, the authors have simply copied out their index cards after rough classification. Incompatible conclusions appear side by side without comment, though some reconciliation is attempted in a 17-page introductory survey.

Triticale can be made by crossing several subspecies of wheat and rye; it is therefore a genus rather than a species. Sterile crosses were made in 1875, colchicine-induced polyploidy made them partially fertile in 1937. Since then many fully fertile strains have been produced, compared and standardised. In comparable conditions the better strains seem to give larger yields than wheat; an experiment is quoted in which triticale yielded 8.3 and wheat 7.2 tons per hectare. As with wheat and rye, the protein content of the grain varies with cultural conditions, but triticale seems usually to surpass the other two. The amino acid analyses quoted are by no means concordant. Before the statement that triticale contains more lysine than wheat is accepted, we should be told how many analyses were done on each sample, how many samples from the same crop were taken, and how amino acid composition is affected by cultural conditions. Amino acid analysis is not such a foolproof process as it is often assumed to be.

Many of the properties of triticale are intermediate between those of its parents. It contains less resorcinol than rye and is less susceptible to ergot, but it is not as suitable for bread-making as wheat though a mixture of flours is satisfactory. There seems to be no evidence on the phytic acid or trypsin

inhibitors in triticale; in spite of this, the authors devote four pages to these components of wheat and rye. Triticale is a new crop on which much more research and development are needed. Its superiority to its parents is not yet unequivocally established but is probable. It seems clear that triticale is potentially a valuable crop: someone ought to write a book specifically about it.

N. W. PIRIE

Polar humans

Polar Human Biology. (Proceedings of the SCAR/IUPS/IUBS Symposium on Human Biology and Medicine in the Antarctic.) Edited by O. G. Edholm and E. K. E. Gunderson. Pp. xii+443. (Heinemann: London, November 1973.) £6.00.

THE symposium from which this book resulted took place in Cambridge during September, 1972. The editors are to be congratulated on producing it within a reasonable time and the publishers have provided an attractive format and a price which is not excessive. Certainly, the book deserves to be read widely, for it describes the state of the art of research into polar human biology and medicine at a time when great advances have been and are being made.

The book falls naturally into five sections. An initial series of reviews of the individual contributions to Antarctic research by the several countries concerned is followed by two papers on dental problems and a larger section on virological and immunological research. Next follow a group of papers on various aspects of thermal adaptation, activity patterns and circadian rhythms. The book ends with a large section devoted to psychological and social problems of life under the severe conditions which constitute the polar environment.

As might be expected with such a large range of subjects, the treatment is very uneven. Several of the papers are either anecdotal or present largely unanalysed sick-report data. Though of practical interest they seem out of place here. On the other hand a few papers seem destined to become classics in the study of man at extreme latitudes. In particular the two papers of Shephard and his collaborators Godin and Rode seem to me to combine all that is excellent in a multidisciplinary study of the response of the Eskimo to his rapidly changing pattern of life. Roger's paper on cold adaptation in members of the Commonwealth Trans-Antarctic Expedition of 1958 is equally admirable—indeed, perhaps more so, since the conditions of work must have been much more severe. His conclusion—that general biological adaptation to severe cold does not occur—is interesting to say the least!

The importance of psychological and cultural factors in polar adaptation is rightly stressed. Much of it is of an applied nature, in particular for personal selection, and several of the papers reflect this interest. Again, though, one wonders if this book is the right vehicle for such reports.

Though the book contains much that is good and some things which are really excellent, it is impossible to escape the general conclusion that the international organisation of polar (and particularly Antarctic) research in human biology is in a pretty anarchic state. A good many papers show considerable areas of overlap of topic and similarity of methodology and it seems sad that the impetus of the International Biological Programme towards cooperation and standardisation seems to have petered out in the far south. I hope, though, that this book will be a spur to the greater integration of research. If this happens the enormous devotion and self sacrifice of many of the scientists, working often in an environment as lethal as any in the world, will be fully rewarded.

E. J. CLEGG

Biological information

Elementary Principles of Probability and Information. By R. F. Wrighton. Pp. viii+91. (Academic: London and New York, January 1974.) £1.95; \$5.50.

THE author is primarily concerned with the applications of statistical inference and information theory to the biological sciences. He complains that biologists are 'currently witnessing a resurgence of anthropomorphism as information notions percolate into biological enquiry conceived at the molecular level'. He foresees that present macro-theories, derived essentially from 'purely subjective interpretations of probability' and applied to information processes of macro-systems in which it is always possible to distinguish between 'the label and the labelled', will break down at or near the molecular level.

At first sight, his programme seems to imply a direct though difficult reduction of biology to physics. But the programme is even more fundamental than that. In his last chapter, noting that anthropomorphic heresies, in the form of traces of communication notions, pervade both quantum and relativity theories, the author looks forward to a 'complete fusion of information theory with quantum theory rather than to any less intimate association'. He is therefore claiming that the much closer physical analysis he seeks of biological processes could solve some of the epistemological problems of modern physics. That is an ambitious programme. Is it possible for man to establish a science from which all anthropomorphic residues

Corrosion of metals research 1924-1968

This book, written in a semi-popular style, by Dr Wormwell of the National Physical Laboratory, is intended for people with an interest in corrosion and its prevention. It is a modest contribution to the history of government science and offers an example of what can be achieved by a long-maintained team working in one broad area of endeavour.

£1.65 (£1.72)

A code of practice for the detailed statement of accuracy

The main purpose of this Code of Practice, prepared by the National Physical Laboratory, is to put forward recommendations as to how uncertainty can be expressed so as to avoid ambiguity, and discusses some of the ways in which estimates of uncertainty can be derived from individual measurements.

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are completely eliminated? If so, then at least we have to begin by identifying those residues that can be discerned and considering what might be done about them. And that is what the author attempts to do.

Unfortunately, the reader finds himself embarking on this fundamental programme without being clearly informed about where he is being led. The seven chapters discuss familiar topics in an unfamiliar way—randomness, the basic Shannon model, noise, inverse problems in probability, sampling techniques, and so on. These topics, dealt with in terms of elementary mathematics, enable the author to air the serious problems he has in mind. But it is only slowly and with much re-reading that the reader builds up any coherent picture of the author's objectives. So it is difficult to see to what readers this book is addressed. Those seeking techniques for immediate practical application will find none: those interested in the fundamental problems discussed will be misled by the title and would not expect so serious a programme to be presented in this form.

But the author's ideas are stimulating. Information concepts derived from telecommunications and other physical systems do seem to be inadequate for enquiry into the processes of 'information transfer' on which all living organisms depend. B. C. BROOKES

matters arising

Temperature cycle in the North Pacific

SIR,—I suggest that the evidence presented by Favorite and McLain¹ for trans-Pacific displacement of temperature anomalies at the ocean surface, is far from conclusive and that there is no evidence that waves progress around the North Pacific gyre.

The eastward advance of the anomalies across the Pacific Ocean from 1954 to 1960 is not immediately obvious or orderly. An explanation of the widespread warming in 1955 of the eastern Pacific between 35°N and 40°N, in terms of an eastward displacement of the maximum, implies a rate of advance of some 15 km d⁻¹. This is about three times greater than the generally accepted pattern of geostrophic flow, which is slightly more than 5 km d⁻¹. Similarly, the rate of advance of the positive anomaly from the western side to the eastern side of the ocean from 1956 to 1957 would have been some 15–20 km d⁻¹. Conversely, there was very little change from 1958 to 1960, when, according to the proposed hypothesis, the negative anomaly should have progressed eastwards to embrace the western coast of North America. Another discrepancy is the westward movement of the negative anomaly in the eastern Pacific from 1955 to 1956.

If the rate of advance of the temperature anomalies, indicated by geostrophic calculations, is slightly more than 5 km d⁻¹ and the circumference of the North Pacific gyre is some 18,000 km, then the time required for a water parcel to complete one circuit would be nearer 9 yr than the 6 yr suggested.

Suggestions that there is evidence of

the temperature cycle as far back as 1930 in the western subarctic region carry little weight in view of the statement that "it is difficult to establish normal conditions at any time or place that would allow the detection of anomalous conditions in a water column at the western side of the ocean."

A simpler explanation of the 5–6 yr cycle in sea surface temperature anomalies in the North Pacific is that heating and cooling occur *in situ*. Baur² has shown that the intensity of the solar beam undergoes a double systematic variation, amounting to 0.5%, within the 11-yr sunspot cycle, with primary maximum strength at about 0.4 of the rising phase and a secondary maximum at about 0.6 of the declining phase of solar disturbance; a sharp minimum of the solar constant occurs just before sunspot minimum, with a secondary minimum at about 0.6 of the rising phase. These variations of the solar constant have been applied to the known years of sunspot extremes from 1950 to 1968 (ref. 3). Figure 1 shows that there is a high positive correlation between sea surface temperature anomalies in the North Pacific, and fluctuations of the solar constant.

There are other well documented relationships between variations in the output of solar energy and meteorological and climatological phenomena. The height of the tropopause at Leopoldville, near the equator, rose by more than 1 km between the 1954 solar minimum and the 1957 maximum (ref. 4). This could be interpreted as resulting from a strengthening of the ascending branch of the Hadley cell associated with greater energy transfers in the atmosphere at the time of increasing output of solar radiation. In East Africa, the level of Lake Victoria was highest in 1952, 1957 and 1964, some 1–2 yr after maxima³ of the solar constant. Again, an increase in surface heating at the time of maximum solar output would lead to an increase in convection and thus to an increased tropical rainfall. In European Russia there were sharp fluctuations in the mean temperature from month to month at times when solar activity was increasing in 1956–57⁵ and in 1967–69⁶. The strength of the westerlies over New Zealand tended to increase about 10 months after rises of solar activity (B. N. Parker, unpublished information). More generally, large fluctuations of Baur's solar index⁶ in the extreme sunspot

cycles since about 1940 coincide with decades of increased variability of wind circulation, with falling values of indices of the strength of the zonal windstreams and with increasing variance of climatic elements in many parts of the world³. Processes by which solar activity may influence weather and climate are discussed by Lamb³.

Thus, the 5–6 yr temperature cycle in the North Pacific could possibly be caused by systematic variations of surface heating resulting from variations in the output of solar energy during the 11-year sunspot cycle. This simple pattern would be modified by ocean-atmosphere interactions and quasistationary waves, to produce regional variations in the timing of the maxima and minima. The geographical distribution of temperature anomalies at each phase of the sunspot cycle would then not be expected to be the same in each cycle. In fact the coherence evident during the 1958–60 cycle was not readily apparent during 1948–52 or 1961–67 (ref. 1). It is to be expected that the effects of these variations in the output of solar energy should also be evident in temperature anomalies in other oceans.

Yours faithfully,
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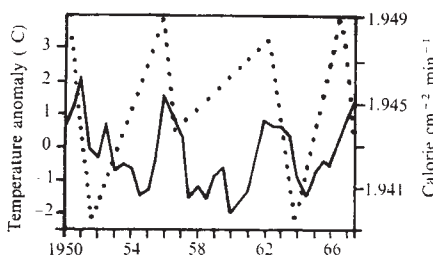


FIG. 1 — semiannual anomalies of mean sea surface temperature in Marsden square 163, quadrant 1 (40–45°N, 170–175°E) from 20 yr mean, 1948–67 (from ref. 1). Average variation of solar constant from phase to phase of the 11 yr sunspot cycle² applied to the sunspot extremes³.

Macromolecular structures for undergraduates

MEYER¹ and Feldman *et al.*² describe the use of computer displays for the three-dimensional study of macromolecular structures. They suggest that, in the near future, such systems will be available for teaching purposes. As students we should like to point out that computer graphics have disadvantages for teaching in England, and that there are now available molecular models which may be used to emphasise different aspects of structure in an immediately

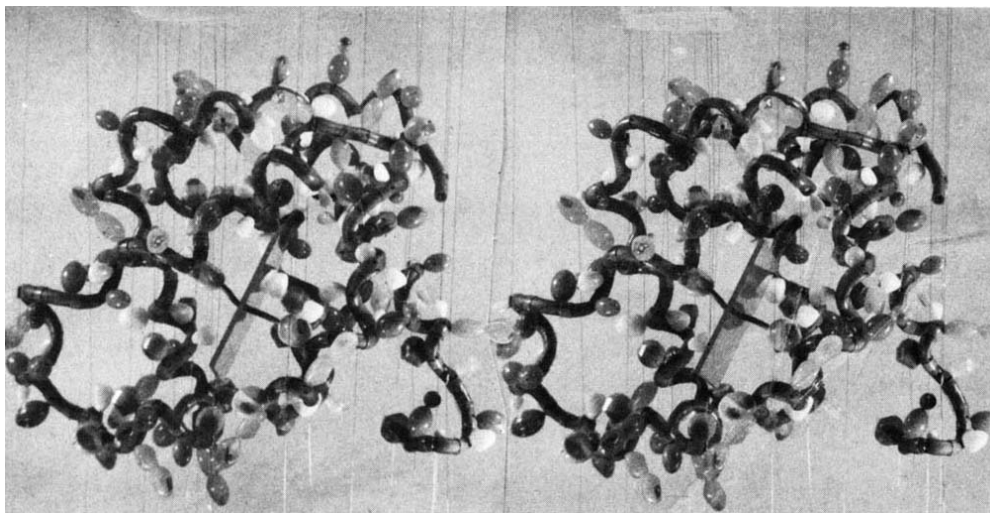


FIG. 1 Myoglobin stereopair built from Biobits model system. The helices, N- and C- terminal ends, haem pockets, types of R groups and their location on the surface or within the interior of the molecule are readily and immediately obvious.

comprehensible manner. One of these systems³ in particular makes possible the building of a complete macromolecular structure such as myoglobin within a framework 60 × 60 × 30 cm (Fig. 1) and in one to two days.

One problem in the use of computer graphics is the cost of installing and running the apparatus. Another is that students would need specialised training to use it. Models have the advantages that they are built by the student, can be handled and viewed from all sides, and are portable. Models display more than adequately for the undergraduate the features of families of macromolecules: there seems little point in listing the features of molecule after molecule in a computer display.

Biobits models for proteins and nucleic acids are available from Capital

Biotechnic Developments Ltd., 66A Churchfield Road, London W3, United Kingdom, and from Ealing Corporation, 2225 Massachusetts Avenue, Cambridge, Massachusetts 02140. With these models we have been able to build accurate scale models of myoglobin, carboxypeptidase A, LDH monomer and other proteins within a day or two of receiving the X-ray data. Indeed we have probably built and studied a wider range of proteins than any other undergraduates in England including those in the major X-ray crystallographic departments.

We appreciate the tremendous value of computer graphics: the system can, for example, overlay structures of two molecules for direct comparison in a manner which models could never do. But we hope that before any depart-

ment switches to this complex and expensive system it will reconsider the very great advantages of real models.

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Announcements

International meetings

May 1-5, **22nd Annual Colloquium on Protides of the Biological Fluids** (XXIInd Colloquium on Protides of the Biological Fluids, Simon Stevin Instituut, Jerusalemstraat 24, B8000 Brugge, Belgium)

May 5-9, **ECBO Study Group on Ageing: Symposium on Impairment of Cellular Functions during Ageing in vivo and in vitro** (Dr E. Holečková, Czechoslovak Academy of Sciences, Institute of Physiology, Budějovická 1083, 142 20 Prague, Czechoslovakia)

May 6-8, **International Water Pollution Conference** (Conference Secretariat (EEC Amsterdam), Society of Chemical Industry, 14, Belgrave Square, London SW1X 8PS, U.K.)

May 6-10, **First International Mercury Congress** (Secretaria del Congreso,

Facultad de Ciencias (Pedralbes) Barcelona 14, Spain)

May 8-10, **Minicomputers in Data Communication** (Penny Green, Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS, U.K.)

May 8-11, **Management Techniques for Metallurgists** (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N 20 9LW)

May 14, **Symposium on Cancer—The Patient and Family** (Mr P. A. Sturgess, Assistant Secretary, Marie Curie Memorial Foundation, 124 Sloan Street, London SW1X 9BP)

May 14-16, **Conference on Neurobiology of CNS Hormone Interactions** (Dr Walter E. Stumpf and Dr Lester D. Grant, 111 Swing Building, University of North Carolina, Chapel Hill, North Carolina 27514)

May 14-18, **West European Conference on Marine Technology** (The Royal In-

stitution of Naval Architects, 10 Upper Belgrave Street, London SW1X 8BQ)

May 14-22, **US-ROC Seminar on Plant Tissue and Cell Cultures** (Dr L. G. Nickell, Hawaiian Sugar Experiment Station, Hawaii)

May 15-17, **3rd International Conference on the Hydraulic Transport of Solids in Pipes** (Organising Secretary, Hydrotransport 3, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, U.K.)

May 16, **Friction, Wear and Lubrication** (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX)

May 16-18, **Physiology of the Nephron: Mechanism and Regulation** (Secretariat Organizing Committee, (Dr J. P. Bonvalet), Unite de Recherches de Pathologie Cardiovasculaire, I.N.S.E.R.M., Hôpital Leon Bernard, 94450 Limeil Brevannes, France)